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RENAL FUNCTION IN PERNICIOUS ANEMIA

AS DETERMINED BY DIETARY RENAL TESTS *

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BOSTON

In 1914 Hedinger and Schlayer[1] described a dietary test for renal function which consisted in estimating the amount of urine, its specific gravity, and its content in sodium chlorid and nitrogen, both the total amount and percentage concentration in two hour specimens collected throughout the day and in a single night specimen. Their patients were placed on a special diet so arranged that different meals given throughout the day should have varying amounts of fluids, sodium chlorid, protein and purin bases. Slight modifications of these diets will be found in the papers published by Mosenthal[2] from the Johns Hopkins Hospital Clinic, and O'Hare[3] from the Peter Bent Brigham Hospital Clinic.

A normal person under the conditions of such a diet shows variations in all of the factors determined in the test in relation to the meals, so that when the results of the test are charted the columns and lines form an irregular or picket fence curve (Fig. 1, P. B. B. H. Med. No. 3337). In cases of nephritis these curves are flattened out toward straight lines in relation to the character and severity of the renal involvement (Fig. 2, P. B. B. H. Med. No. 2567). Mosenthal[2] mentions that in patients with severe anemia results were obtained with the test diet which are similar in every detail to those found in advanced cases of contracted kidney. Similar conditions he finds in patients with prostatic hypertrophy, pyelonephritis and polycystic kidney. In these

* Submitted for publication July 3, 1916.
* From the Medical Clinic of the Peter Bent Brigham Hospital.
* Read by title at a meeting of the American Society for the Advancement of Clinical Investigation, held in Washington, D. C., May 8, 1916.

1. Hedinger and Schlayer: Deutsch. Arch. f. klin. Med., 1914, cxiv, 120.
2. Mosenthal: The Archives Int. Med., 1915, xvi, 733.
3. O'Hare: The Archives Int. Med., 1916, xvii, 711.

latter it would seem that pathological conditions simulate closely those found in chronic nephritis, whereas in severe anemia it would not be probable that the kidney had undergone a change in any way analogous to the changes of chronic nephritis. Mosenthal[4] gives one chart of a patient with anemia in which the hemoglobin was 44 per cent. and the red blood cells 2,240,000, but he gives no further details as to the patient's condition. It seemed interesting in view of this report of

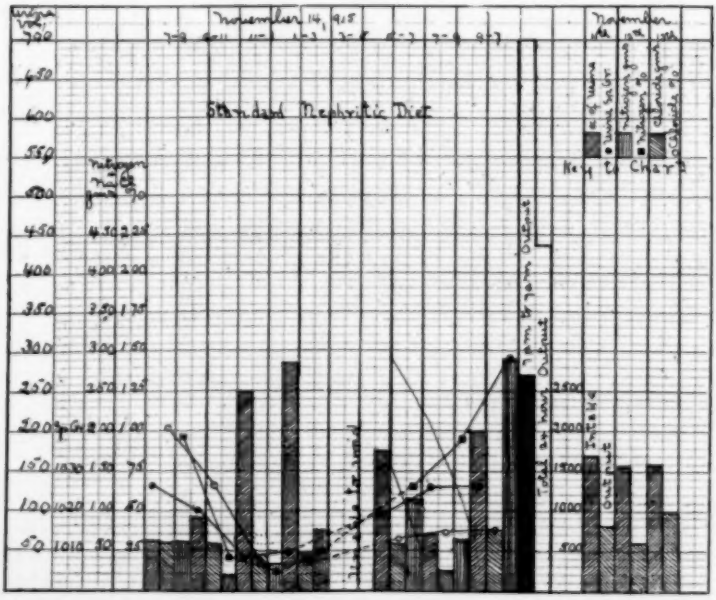

Fig. 1 (P. B. B. H., Med. No. 3337).—Diagnosis, mitral insufficiency and regurgitation; chronic myocarditis. The heart at time of the test was well compensated. In this and the following three illustrations, the series of columns beginning at the left give the amount of urine, sodium chlorid and nitrogen in every two-hour portion from 7 a. m. to 9 p. m., and in the portion from 9 p. m. to 7 a. m. The solid lines joining dots in the space of each column give the specific gravity of the urine and the percentage concentration of sodium chlorid and nitrogen. Next is indicated the night portion of urine in relation to the total twenty-four-hour amount. The columns at the right give the twenty-four-hour fluid intake and urine output in the days preceding the test day.

4. Mosenthal in a recent paper before the medical section of the American Medical Association, held in Detroit, June 13-15, 1916, has given a further discussion of these changes in renal function in cases of severe anemia and outlined the results of examination of several cases.

Mosenthal to test out a series of cases of pernicious anemia, and this we have done.

Each of the cases reported below had the blood picture typical of pernicious anemia and in every way conformed to that diagnosis, so that it hardly seems necessary to give in detail the clinical findings in these cases. Instead, simply the blood and urine findings will be stated in relation to the time of the dietary test of renal function and the results of the dietary tests of renal function will be given in the form of tables and charts.

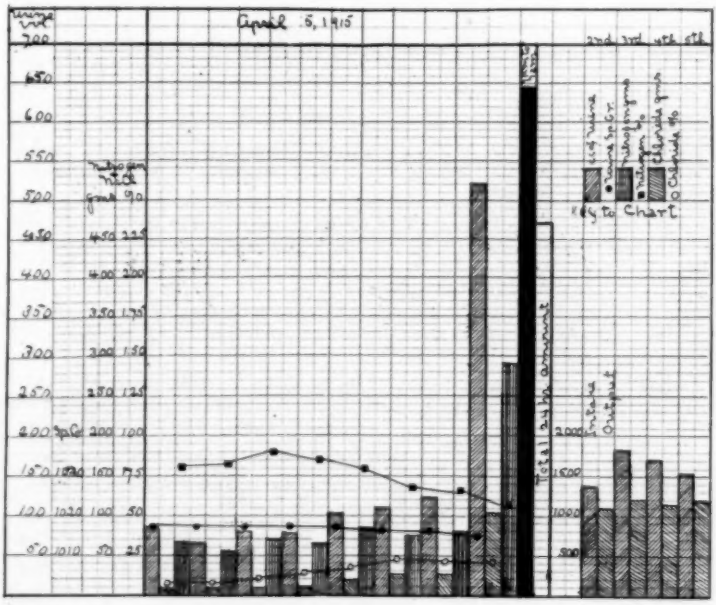

Fig. 2 (P. B. B. H., Med. No. 2567).—Diagnosis, chronic nephritis and hypertension. (For the key to this figure see Figure 1.)

Case 1 (P. B. B. H. Med. No. 4318).—A man, aged 46, on blood examination made March 19, showed hemoglobin 19 per cent.; red blood corpuscles 1,420,000; on March 27, hemoglobin 27 per cent.; red blood corpuscles 1,100,000. The phenolsulphonephthalein excretion April 5 was 72 per cent. in two and one-quarter hours.

The urine on March 20 showed no albumin, but an occasional hyaline and granular cast; on April 30 there were no albumin or casts.

As treatment on February 25 diarsenol was injected intravenously, 0.2 gm.; on March 9, 0.3 gm., and on March 14 there was transfusion of 750 c.c. blood.

TABLE 1.—Two-Hour Renal Test in Case 1, Made March 24, 1916

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9	119	1.019	0.58	0.69	0.30	0.36
9 to 11						
11 to 1	122	1.018	0.65	0.79	0.30	0.37
1 to 3	123	1.018	0.75	0.92	0.29	0.36
3 to 5	122	1.017	0.61	0.74	0.28	0.34
5 to 7	135	1.016	0.60	0.83	0.25	0.35
7 to 9	149	1.017	0.60	0.89	0.21	0.31
9 to 7	410	1.019	0.71	2.91	0.25	1.03
Total	1,183	.....	....	7.77	....	3.12

A blood test April 9, in Case 1 showed hemoglobin 19 per cent., and red blood corpuscles 970,000; and on April 12, hemoglobin 16 per cent., and red blood corpuscles 920,000.

The urine on April 10 showed no albumin and no casts.

On April 8 transfusion of 300 c.c. blood was made.

TABLE 2.—Two-Hour Renal Test in Case 1, Made April 11, 1916

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9	123	1.016	0.46	0.57	0.52	0.64
9 to 11	160	1.015	0.37	0.59	0.54	0.86
11 to 1	92	1.016	0.48	0.44	0.56	0.53
1 to 3	153	1.015	0.40	0.61	0.54	0.83
3 to 5	150	1.014	0.41	0.62	0.50	0.75
5 to 7	71	1.016	0.54	0.38	0.48	0.34
7 to 9	135	1.014	0.60	0.81	0.46	0.62
9 to 7	475	1.014	0.48	2.28	0.44	2.09
Total	1,359	.....	....	6.30	....	6.66

On April 12 blood test showed hemoglobin 16 per cent., red blood corpuscles 970,000; on April 14, hemoglobin 35 per cent., red blood corpuscles 1,320,000.

On April 12 a transfusion of 600 c.c. blood was made.

TABLE 3.—Two-Hour Renal Test in Case 1, Made April 14, 1916*

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9	245	1.015	0.32	0.78	0.86	2.11
9 to 11	92	1.019	0.23	0.21	0.99	0.90
11 to 1	86	1.020	0.54	0.46	0.98	0.84
1 to 3	117	1.019	0.48	0.56	0.91	1.07
3 to 5	135	1.017	0.55	0.74	0.76	1.03
5 to 7	125	1.018	0.54	0.68	0.79	0.99
7 to 9	215	1.016	0.38	0.82	0.68	1.86
9 to 7	400	.....	0.38	0.82	0.68	1.86
Total	1,415	.....	....	4.04+	....	8.80+

* In the table of April 14, in contrast to that of April 11, there is a slightly less marked fixation in curves of excretion along with the moderately improved blood condition following transfusion.

Case 2 (P. B. B. H. Med. No. 4181).—A man, aged 51 years, was given a blood test April 11, and showed hemoglobin 42 per cent., red blood corpuscles 1,960,000.

The phenolsulphonephthalein excretion on April 5 was 66 per cent. in two and one-quarter hours. The urine on April 8, showed no albumin and no casts.

On February 29 a transfusion of 550 c.c. blood was made. On April 6 diarsenol was given intravenously, 0.2 gm., and on April 9 another transfusion of 375 c.c. blood was made.

TABLE 4.—Two-Hour Renal Test in Case 2, Made April 11, 1916

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9	48	1.026	0.78	0.37	1.28	0.61
9 to 11	75	1.025	1.07	0.80	0.96	0.72
11 to 1	64	1.024	0.95	0.61	0.99	0.63
1 to 3	74	1.024	1.04	0.77	0.98	0.73
3 to 5	100	1.024	0.82	0.82	0.98	0.98
5 to 7	98	1.025	0.70	0.69	0.98	0.96
7 to 9	87	1.023	0.85	0.74	0.97	0.87
9 to 7	448	1.019	0.56	2.51	0.98	4.39
Total	994	.....	....	7.31	....	9.89

Blood examination in Case 2, made April 27, showed hemoglobin 80 per cent., red blood corpuscles 3,460,000; and on May 4, hemoglobin 82 per cent., red blood corpuscles 3,500,000.

The urine April 28, showed no albumin or casts.

On April 18, diarsenol was given intravenously, 0.2 gm.

TABLE 5.—Two-Hour Renal Test in Case 2, Made May 3, 1916*

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9	76	1.022	0.63	0.48	1.26	0.96
9 to 11	67	1.024	0.64	0.43	1.23	0.82
11 to 1	130	1.019	0.50	0.65	0.99	1.29
1 to 3	104	1.024	0.73	0.76	1.07	1.11
3 to 5	128	1.022	0.64	0.82	1.16	1.49
5 to 7	56	1.028	0.92	0.52	1.38	0.72
7 to 9	74	1.027	0.90	0.67	1.26	0.93
9 to 7	603	1.017	0.56	3.38	0.88	5.31
Total	1,238	.....	....	7.71	....	12.63

* These two observations on the same case show evidence of a return toward normality in renal excretion with improved blood condition.

Case 3 (P. B. B. H., Med. No. 4292).—A woman, aged 28 years, was given a blood examination April 13, which showed hemoglobin 37 per cent., red blood corpuscles 1,230,000. The phenolsulphonephthalein excretion on April 13 was 36 per cent. in two hours.

The urine on April 8 showed the slightest possible trace of albumin, but large numbers of hyaline and finely granular casts, and an occasional coarsely granular cast.

The treatment from March 9 to March 17, was Fowler's solution and hydrochloric acid. On March 15 transfusion of 500 c.c. blood was made, and on April 10 diarsenol, 0.2 gm., was given intravenously.

TABLE 6.—Two-Hour Renal Test in Case 3, Made April 17, 1916

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9	200	1.012	0.24	0.48	0.52	1.04
9 to 11	660	1.007	0.03	0.20	0.19	1.25
11 to 1						
1 to 3	205	1.012	0.13	0.27	0.47	0.96
3 to 5	380	1.011	0.06	0.23	0.28	1.44
5 to 7	Unable to void					
7 to 9						
9 to 7						
Total	1,445	.....	....	1.18	....	4.69

Blood test made April 25 showed hemoglobin 61 per cent., red blood corpuscles 1,780,000, and on April 29, hemoglobin 76 per cent., red blood corpuscles 1,840,000.

The treatment from April 24 to April 26 was Fowler's solution, and on April 26 diarsenol was given intravenously, 0.2 gm.

TABLE 7.—Two-Hour Renal Test in Case 3, Made April 26, 1916

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9	245	1.014	0.38	0.69	0.63	1.54
9 to 11	275	1.008	0.15	0.41	0.27	0.64
11 to 1	240	1.007	0.13	0.31	0.27	0.65
1 to 3	180	1.012	0.15	0.27	0.56	1.01
3 to 5	310	1.008	0.14	0.43	0.38	1.18
5 to 7	250	1.010	0.22	0.55	0.52	1.30
7 to 9 9 to 7	185	1.013	0.11	0.20	0.51	0.92
Total	1,685	.....	....	2.86	....	7.24

Case 4 (P. B. B. H., Med. No. 4316).—A woman, aged 48, was given a blood examination March 13, which showed hemoglobin 52 per cent., red blood corpuscles 1,720,000; and on March 20, hemoglobin 54 per cent., red blood corpuscles 1,810,000. The phenolsulphonephthalein excretion on April 7 was 57 per cent. in two hours.

The urine on March 15 showed very slight trace of albumin and few hyaline and granular casts. On April 4 there was the slightest possible trace of albumin with an occasional hyaline and granular cast.

The treatment was Fowler's solution March 14 to March 23.

TABLE 8.—Two-Hour Renal Test in Case 4, Made March 16, 1916

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9	56	1.019	0.46	0.26	0.44	0.25
9 to 11 11 to 1	120	1.018	0.74	0.89	0.30	0.36
1 to 3 3 to 5	160	1.020	0.73	1.17	0.36	0.58
5 to 7	60	1.019	0.77	0.46	0.36	0.22
7 to 9 9 to 7	307	1.020	0.42	1.29	0.34	1.04
Total	703	.....	....	4.07	....	2.45

Case 5 (P. B. B. H., Med. No. 4404).—A woman, aged 60, was given a blood examination March 27, which showed hemoglobin 30 per cent., red blood corpuscles 1,440,000; and on April 3, hemoglobin 34 per cent., red blood corpuscles 1,430,000. The phenolsulphonephthalein excretion on April 7 was 55 per cent. in two hours.

The urine on March 29 showed the slightest possible trace of albumin and few hyaline and granular casts. On April 8 there was the slightest possible trace of albumin with an occasional hyaline and granular cast.

The treatment was Fowler's solution from March 28 to April 10.

TABLE 9.—Two-Hour Renal Test in Case 5, Made March 31, 1916

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9	140	1.016	0.74	1.05	0.42	0.59
9 to 11	33	1.016	0.44	0.15	0.38	0.13
11 to 1	134	1.017	0.71	0.95	0.38	0.51
1 to 3						
3 to 5	46	1.018	0.38	0.17	0.34	0.16
5 to 7	78	1.015	0.71	0.52	0.40	0.29
7 to 9	56	1.016	0.71	0.40	0.31	0.17
9 to 7						
Total	482	.....	....	3.24	....	1.85

Case 6 (P. B. B. H., Med. No. 4217).—A woman, aged 60, was given a blood examination March 10, which showed hemoglobin 26 per cent., red blood corpuscles 790,000; and on March 17, hemoglobin 26 per cent., red blood corpuscles 750,000. The phenolsulphonephthalein excretion on March 7 was 36 per cent. in two hours.

The urine on March 16 showed very slight trace of albumin with an occasional hyaline and granular cast.

This patient had had splenectomy several months before with but little resultant change in the blood picture. Later there had been a decline in the blood condition.

TABLE 10.—Two-Hour Renal Test in Case 6, Made March 15, 1916

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9	39	1.016	0.64	0.25	0.28	0.11
9 to 11	36	1.016	0.72	0.26	0.28	0.10
11 to 1	101	1.014	0.69	0.70	0.28	0.28
1 to 3	78	1.014	0.35	0.27	0.29	0.23
3 to 5	102	1.014	0.52	0.53	0.29	0.30
5 to 7						
7 to 9	30	1.018	0.17	0.03	0.33	0.07
9 to 7	400	1.014	0.64	2.56	0.36	1.44
Total	776	.....	....	4.00	....	2.53

Case 7 (P. B. B. H., Med. No. 4505).—A man, aged 47, was given a blood examination April 13, which showed hemoglobin 45 per cent., red blood corpuscles 1,970,000; on April 18, hemoglobin 48 per cent., red blood corpuscles

1,300,000; and on April 25, hemoglobin 49 per cent., red blood corpuscles 1,420,000. The phenolsulphonephthalein excretion on April 14 was 67 per cent. in two hours.

The urine on April 15 showed no albumin and the sediment was negative. The same condition existed on April 25.

TABLE 11.—Two-Hour Renal Test in Case 7, Made April 18, 1916

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9	45	1.024	0.73	0.33	0.22	0.10
9 to 11	95	1.021	1.07	1.02	0.44	0.42
11 to 1	87	1.024	1.03	0.90	0.66	0.57
1 to 3	66	1.024	1.15	0.76	0.63	0.42
3 to 5	165	1.022	0.79	1.30	0.78	1.29
5 to 7	110	1.023	0.68	0.75	0.96	1.06
7 to 9	66	1.023	0.90	0.65	0.52	0.34
9 to 7	150	1.023	1.12	1.68	0.72	1.08
Total	784	.....	....	7.39	....	5.28

Case 8 (P. B. B. H., Med. No. 4064).—A man, aged 63 years, was given a blood examination March 17, which showed hemoglobin 35 per cent., red blood corpuscles 1,520,000; and on March 26, hemoglobin 41 per cent., red blood corpuscles 1,728,000. The phenolsulphonephthalein excretion on April 5 was 38 per cent. in two and one-quarter hours.

The urine on March 23 showed the slightest possible trace of albumin and no casts. The same condition was shown on April 3.

The treatment from January 30 to February 5 was Fowler's solution. On February 12 diarsenol, 0.3 gm. was given intravenously. The same amount was given on February 26, and on March 8 a transfusion of 750 c.c. of blood was made.

TABLE 12.—Two-Hour Renal Test in Case 8, Made March 23, 1916

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9	107	1.009	0.47	0.50	1.02	1.09
9 to 11	75	1.022	0.62	0.47	0.91	0.68
11 to 1	74	1.021	0.51	0.38	0.95	0.70
1 to 3	100	1.021	0.60	0.60	0.94	0.94
3 to 5	90	1.032	0.40	0.36	1.13	1.02
5 to 7	70	1.021	0.50	0.28	0.90	0.63
7 to 9	92	1.021	0.43	0.40	0.79	0.73
9 to 7	400	1.019	0.36	1.47	1.02	4.17
Total	1,023	.....	....	4.56	....	9.01

Another blood examination was made April 11, which showed hemoglobin 43 per cent., red blood corpuscles 1,510,000; and on April 18, hemoglobin 47 per cent., red blood corpuscles 1,700,000.

The urine on April 18 showed no albumin and no casts.

TABLE 13.—Two-Hour Renal Test in Case 8, Made April 16, 1916*

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9	180	1.020	0.63	1.13	1.08	1.85
9 to 11	106	1.022	0.73	0.77	0.93	0.98
11 to 1	188	1.021	0.61	1.15	0.97	1.82
1 to 3	92	1.022	0.68	0.65	0.97	0.89
3 to 5	118	1.022	0.69	0.68	0.99	1.23
5 to 7	97	1.022	0.65	0.63	1.05	1.02
7 to 9	125	1.020	0.56	0.70	0.89	1.11
9 to 7	515	1.020	0.58	2.99	1.08	5.56
Total	1,415	.....	....	8.70	....	14.46

* Here the two obervations are practically identical and the blood at each observation showed almost the same condition.

Case 9 (P. B. B. H., Med. No. 4609).—A man, aged 73, was given a blood examination May 4, which showed hemoglobin 29 per cent., red blood corpuscles 1,090,000; and on May 8, hemoglobin 31 per cent., red blood corpuscles 1,020,000. The phenolsulphonephthalein excretion on May 8 was 34 per cent. in two hours.

The urine on May 6, showed no albumin, but an occasional hyaline cast. On May 25 there was no albumin, but many leukocytes and epithelial cells.

TABLE 14.—Two-Hour Renal Test in Case 9, Made May 7, 1916

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9	64	1.013	0.43	0.28	0.76	0.49
9 to 11	205	1.013	0.29	0.60	0.67	1.37
11 to 1						
1 to 3	355	1.012	0.25	0.89	0.70	2.49
3 to 5						
5 to 6:30						
6:30 to 9	300	1.009	0.22	0.66	0.34	1.02
9 to 7	435	1.012	0.23	1.00	0.59	2.56
Total	1,359	.....	....	3.43	..	7.93

Case 10 (P. B. B. H., Med. No. 4694).—A man, aged 63, was given a blood examination, May 18, which showed hemoglobin 35 per cent., red blood corpuscles 1,280,000; and on May 25, hemoglobin 34 per cent., red blood corpuscles 1,360,000. The phenolsulphonephthalein excretion on May 22 was 54 per cent. in two hours.

The urine on May 20, showed the slightest possible trace of albumin, with an occasional epithelial cell, hyaline cell and finely granular cast.

The treatment from May 19 to June 6 was dilute hydrochloric acid, given three times a day. On May 24, diarsenol, 0.3 gm., was given intravenously.

TABLE 15.—Two-Hour Renal Test in Case 10, Made May 24, 1916

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9	59	1.018	0.73	0.43	0.52	0.31
9 to 11	79	1.018	0.55	0.42	0.56	0.43
11 to 1	95	1.017	0.63	0.60	0.63	0.60
1 to 3	74	1.018	0.74	0.55	0.59	0.44
3 to 5	53	1.019	0.76	0.40	0.47	0.25
5 to 7	77	1.018	0.83	0.64	0.44	0.34
7 to 9	73	1.017	0.72	0.52	0.40	0.29
9 to 7	213	1.017	0.73	1.56	0.38	0.81
Total	720	.....	....	5.12	...	3.47

Case 11 (P. B. B. H., Med. No. 4478).—A woman, aged 38, was given a blood examination April 8, which showed hemoglobin 28 per cent., red blood corpuscles 1,140,000. The phenolsulphonephthalein excretion on April 10, was 60 per cent. in two hours.

The urine on April 10 showed the slightest possible trace of albumin with an occasional hyaline and finely granular cast.

The treatment was dilute hydrochloric acid, 1 c.c. three times a day from April 8 to May 1.

TABLE 16.—Two-Hour Renal Test in Case 11, Made April 12, 1916

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9	39	1.018	0.84	0.33	0.40	0.16
9 to 11	48	1.016	0.74	0.26	0.28	0.15
11 to 1 / 1 to 3	135	1.012	0.68	0.92	0.34	0.46
3 to 5	82	1.012	0.71	0.58	0.24	0.20
5 to 7	175	1.009	0.42	0.74	0.18	0.32
7 to 9	101	1.013	0.52	0.53	0.28	0.28
9 to 7	453	1.013	0.44	1.99	0.30	1.36
Total	1,033	.....	....	5.35	....	2.93

Case 12 (P. B. B. H., Med. No. 4748).—A man, aged 42 years, was given a blood examination June 3, which showed hemoglobin 34 per cent., red blood

corpuscles 1,330,000; and on June 9, hemoglobin 40 per cent., red blood corpuscles 1,560,000. The phenolsulphonephthalein excretion on May 30 was 45 per cent. in two hours.

The urine on May 30 showed no albumin, with occasional epithelial cell, but no casts. The same condition existed June 9.

On June 8, 375 c.c. citrated blood was given intravenously.

TABLE 17.—Two-Hour Renal Test in Case 12, Made June 8, 1916

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9	66	1.015	0.57	0.38	0.50	0.33
9 to 11 / 11 to 1	125	1.017	0.69	0.86	0.40	0.50
1 to 3	138	1.015	0.52	0.72	0.42	0.58
3 to 5	370	1.007	0.18	0.68	0.09	0.34
5 to 7 / 7 to 9	204	1.006	0.06	0.12	0.08	0.16
9 to 7	250	1.012	0.68	1.70	0.07	0.18
Total	1,162	.....	....	4.46	....	2.09

Case 13 (P. B. B. H., Med. No. 4565).—A woman, aged 38 years, was given a blood examination April 26, which showed hemoglobin 19 per cent., red blood corpuscles 460,000. The phenolsulphonephthalein excretion on April 28 was 24 per cent. in two hours.

The urine on April 28, showed no albumin, but a few squamous cells. There was no albumin again on May 25, but an occasional coarsely granular cast and epithelial cell.

The treatment was dilute hydrochloric acid, 1 c.c. three times a day from April 26 to May 10.

TABLE 18.—Two-Hour Renal Test in Case 13, Made April 27, 1916

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9 / 9 to 11 Unable to void						
2 a. m. to 12:20	515	1.012	0.50	2.58	0.02	0.10
12:20 to 3 Unable to void						
3 to 5	310	1.012	0.46	1.43	0.04	0.12
5 to 7 / 7 to 9 Unable to void						
9 to 7	160 at 5 p. m. lost					
Total	985	.....	....	4.01+	....	0.22+

Another blood examination was made April 30, which showed hemoglobin 19 per cent., red blood corpuscles 710,000.

TABLE 19.—Two-Hour Renal Test in Case 13, Made April 30, 1916

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9 9 to 11	15	.....	0.72	0.11	0.06	0.01
11 to 1 1 to 3	405	1.012	0.55	2.24	0.03	0.12
3 to 5 5 to 7 7 to 8	360	1.012	0.58	1.93	0.03	0.10
8 to 7	280	1.012	0.47	1.29	0.47	1.29
Total	1,000	.....	....	5.57	....	1.52

Further examination of the blood on June 3, showed hemoglobin 74 per cent., red blood corpuscles 2,510,000; and on June 11, hemoglobin 70 per cent., red blood corpuscles 2,740,000.

The urine on June 4 showed no albumin, but many epithelial cells and leukocytes, with rarely a coarsely granular cast. On June 15 there was no albumin but some epithelial cells.

On May 6 a transfusion of 600 c.c. of blood was made; also 500 c.c. on May 7, and on May 23, 600 c.c. citrated blood were injected.

TABLE 20.—Two-Hour Renal Test in Case 13, Made June 8, 1916*

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9	170	1.010	0.27	0.46	0.52	0.88
9 to 11	82	1.014	0.43	0.35	0.72	0.59
11 to 1	340	1.007	0.13	0.44	0.26	0.88
1 to 3	66	1.019	0.61	0.40	0.94	0.62
3 to 5	160	1.016	0.39	0.62	0.85	1.36
5 to 7	300	1.009	0.27	0.74	0.29	0.87
7 to 9	775	1.007	0.19	0.90	0.30	1.43
9 to 7	400	1.016	0.40	1.60	1.10	4.40
Total	1,983	.....	....	5.51	....	11.03

* In the observations of April 27 and 30 the tests were not very satisfactory owing to the inability of the patient to void at regular intervals and to the small amount of food she was taking. On June 8, when the blood picture had changed from hemoglobin 19 per cent. and red blood corpuscles 460,000 on April 26 to hemoglobin 74 per cent. and red blood corpuscles 2,510,000 on June 3, following treatment, the observations on renal function showed a marked return toward normality.

A glance at the tables and illustrations shows a very evident tendency to fixation in the curve of the specific gravity. Similarly there is fixation in the percentage concentration of the sodium chlorid, with a much less tendency toward fixation in the percentage concentration of the nitrogen. A number of the patients also show a tendency toward an increased amount of night urine, such as is found in cases of chronic nephritis.

In only one patient with pernicious anemia have we failed to find this type of renal function described above. This was a patient who when studied had relatively little anemia, though the blood showed the morphology typical of pernicious anemia. Her excretion (Fig. 4) of water, sodium chlorid and nitrogen showed no fixation in the forenoon, but in the afternoon there was a tendency to fixation at high levels. This type of excretion we have learned to associate with the very early stages of chronic nephritis, an early hypersensitivity of the kidney, followed by evidence of fatigue in the afternoon. Interestingly enough this particular patient had hypertension and other evidence of early chronic nephritis. The details of the blood picture, renal tests, etc., of this patient follow.

CASE 14 (P. B. B. H., Med. No. 4430).—A woman, aged 60, was given a blood examination March 31, which showed hemoglobin 78 per cent., red blood corpuscles 2,320,000; and on April 2, hemoglobin 84 per cent., red blood corpuscles 2,510,000. The phenolsulphonephthalein excretion on April 3 was 49 per cent. in two hours.

The urine on March 31 showed the slightest possible trace of albumin and a moderate number of hyaline and finely granular casts; the same condition existed on April 2.

The treatment was Fowler's solution from April 2 to April 6.

TABLE 21.—TWO-HOUR RENAL TEST IN CASE 14, MADE APRIL 5, 1916

Time	Volume, C.c.	Specific Gravity	Nitrogen		Chlorid	
			Per Cent.	Grams	Per Cent.	Grams
7 to 9	40	1.026	0.61	0.24	0.62	0.25
9 to 11	620	1.008	0.09	0.56	0.12	0.74
11 to 1	195	1.009	0.17	0.33	0.28	0.55
1 to 3	43	1.024	0.66	0.28	0.58	0.25
3 to 5	160	1.018	0.34	0.54	0.88	1.41
5 to 7	88	1.022	0.54	0.47	0.91	0.80
7 to 9	125	1.022	0.66	0.83	0.88	1.10
9 to 7	320	1.018	0.44	1.45	0.81	2.67
Total	1,601	.....	....	4.70	....	7.77

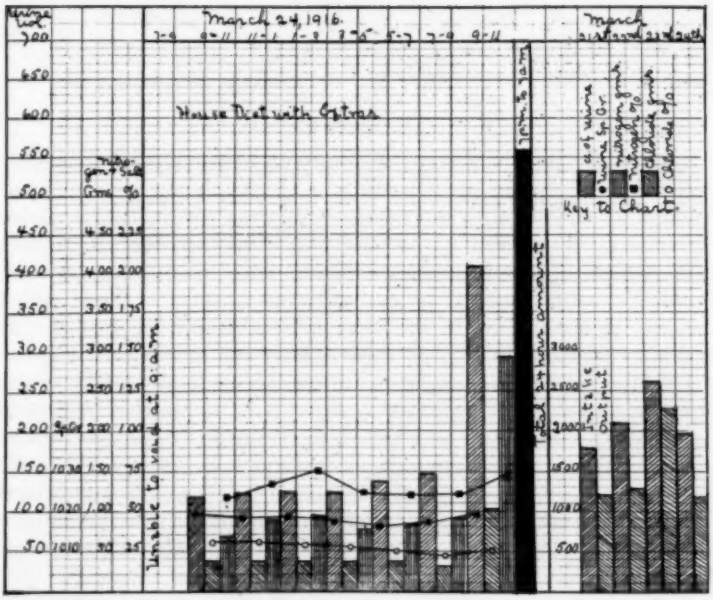

Fig. 3 (Case 1, P. B. B. H., Med. No. 4318).—Diagnosis, pernicious anemia. This figure shows graphically the fixation in the curves of excretion, similar to the condition in Figure 2, from a patient with severe chronic nephritis. (For the key to this figure see Figure 1.)

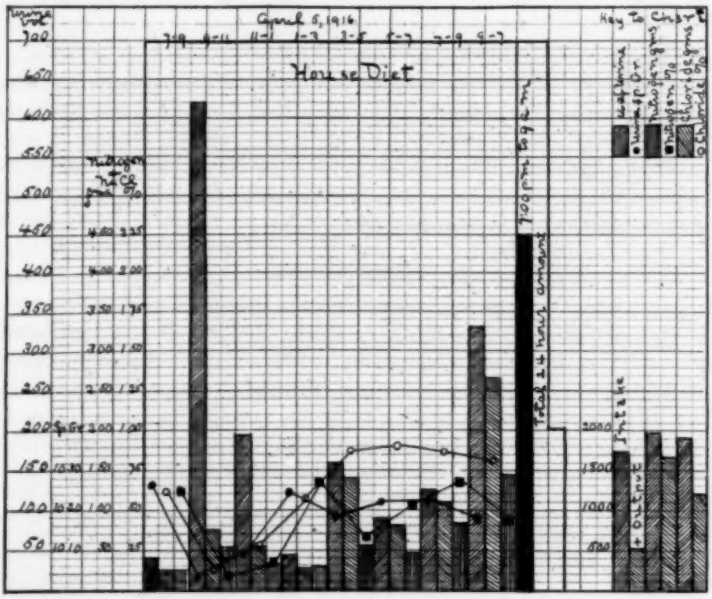

Fig. 4 (Case 14, P. B. B. H., Med. No. 4430).—Diagnosis, pernicious anemia, with very slight anemia and an early stage of chronic nephritis. The illustration shows graphically the lack of fixation in the curves of excretion. The similarity of the curves in Figure 1 from a patient without renal disturbance is quite evident. (For the key to this figure see Figure 1.)

COMMENT

The data given indicates that in a severe anemia renal function, as measured by dietary tests, is disturbed in much the same way as is found in patients with advanced chronic nephritis. In the patients which we have studied are included both young and old. In the latter the element of arteriosclerosis and moderate organic renal lesions of nephritic nature can not be excluded, but in none of these patients except the one in Case 14 was there any evidence in the way of symptoms or physical findings of a renal lesion of the nature of chronic nephritis, and in the younger patients at least the probability of such without symptoms of nephritis is very slight. It seems more probable that this disturbance in excretion is the result of the anemia, either a nutritional or a toxic disturbance in renal cellular activity. This view is supported by the improvement in renal function with improvement in blood condition and by the absence of fixation in Case 14, in which anemia was very slight and the excretion picture very different from the other cases.

Inasmuch as in pernicious anemia the dietary renal test gives a picture similar to that in quite advanced nephritis, caution is required in the interpretation of the results of these tests in patients supposed to have nephritis. If a patient with moderate nephritis, as indicated by other tests and by symptoms, happened to have a fairly severe anemia, this latter factor might give to the picture of renal excretion a fixation suggestive of advanced nephritis and lead to a prognostic interpretation which was unjustifiably poor. Consequently, it will be well to consider the element of anemia when drawing conclusions from dietary renal tests applied to patients with nephritis, though it is not probable that this will prove a complicating factor in many cases, for usually the degree of anemia in patients with nephritis is slight, much less than their pallor would suggest.

CONCLUSIONS

In patients with pernicious anemia the disease is accompanied by a disturbance of renal function, as measured by renal dietary tests, which is similar to that found in patients with advanced chronic nephritis. In these patients there is no other evidence of chronic nephritis and the disturbance appears to be due to the anemia, decreasing with the subsidence of the severity of the anemia unless the anemia is maintained so long that a permanent disturbance of renal function ensues.

My thanks are due to Miss Russell and Miss Cate, chemical technicians in the hospital laboratory, for the quantifications made in this series of cases.

AUTOGENOUS DEFIBRINATED BLOOD IN THE TREATMENT OF BRONCHIAL ASTHMA*

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INTRODUCTION

The correct interpretation of results in the treatment of bronchial asthma presents an exceedingly difficult problem. So chronic, so distressing, so intractable is this malady that any new procedure which appeals to the sufferer and carries with it the slightest hope of relief may produce at least a temporary amelioration of the symptoms. We must be careful, therefore, not to fall into the error of overestimating the value of any new treatment. It is only by repeated observations over a long period of time that any conclusive interpretation can be made. However, six successive cases of bronchial asthma responded so favorably, in our opinion, to the repeated subcutaneous injections of autogenous defibrinated blood as to warrant at least a preliminary report of the cases, the treatment and the theoretical considerations that prompted us to employ this method of therapy and that may help to explain the results obtained.

PURPOSE AND SCOPE OF THE WORK

The work reported in this paper was undertaken with certain definite limitations. Only patients with so-called essential bronchial asthma were treated, not those suffering from dyspnea of renal, cardiac or mediastinal origin. Moreover, we eliminated from our work all patients with bronchial asthma in whom a peripheral causal factor like the nasopharynx could be found. No attempt has been made in this paper to summarize the historical data, the innumerable methods of treatment of asthma or the various theories as to its nature and pathogenesis. Our aim is simply to give the results of a method of treatment undertaken in the course of clinical and experimental research in asthma and explained, we believe, on a rational hypothesis.

TECHNIC

Autoserotherapy has been used in effusions into the various serous cavities of the body, in psoriasis and other skin diseases, and in a number of other conditions. In many cases the employment of this method was empirical; in other cases the treatment was not sufficiently intensive to warrant conclusive results. The method we adopted differed from that of other authors.

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In each of our cases phlebotomy was performed by venepuncture, with a sterile needle. From 20 to 30 c.c. of blood were withdrawn and received in a sterile one-ounce flask containing a number of glass beads. The contents were agitated for from five to seven minutes to separate the fibrin. The defibrinated blood was then drawn into a 30 c.c. syringe and immediately injected subcutaneously into the loin of the patient. Ten injections at intervals of about one week were made in each case. Whenever possible blood was withdrawn during an asthmatic attack, but for obvious reasons this was not always feasible. There were no local or immediate general effects following the injections.

REPORT OF CASES

Brief abstracts of the cases and their treatment follow. Only those patients who received ten injections are here reported. The results of the treatment in other cases now still under observation we expect to report in a future publication.

CASE 1.—P. B., a Russian, aged 55, gave a negative family and past history except that he had snuffed tobacco for many years.

His present illness began nine months before, after exposure to rainy and chilly weather. He was suddenly seized with an attack of shortness of breath lasting several hours, during which there was wheezing in his chest, followed by expectoration and relief. Similar attacks occurred with increasing severity and frequency. At times the patient had to sit up in bed at night until an attack would abate. He had had no hemoptysis or other symptoms except for a sensation of oppression in the epigastrium.

The physical examination revealed a well-built man with an asthmatic type of chest. The lungs showed signs of emphysema. The weight was 154 pounds. The auscultatory systolic blood pressure was 140 mm. of mercury and the diastolic pressure was 85 mm. (Tycos). The urine was normal and the Wassermann test was negative.

Treatment at first consisted of intravenous injections of the patient's serum in amounts of 5 to 8 c.c. at weekly intervals. A phlebotomy was done, the blood received in a sterile vessel and kept in the refrigerator an average of eighteen hours. The serum was then injected. In later injections the serum was separated by immediate centrifugalization and given intravenously. Finally, however, we thought it best to use defibrinated blood, and make the injections subcutaneously, a procedure which we carried out in all the subsequent cases. The amounts reinjected varied from 15 to 25 c.c.

The first injection was given May 24, 1915. After the first few injections, the patient felt improved. He progressively gained in weight, reaching 160 pounds in July and 170 early in September. In the latter month, however, he had a mild attack and requested further treatment. Three more injections of 25 c.c. each of defibrinated blood were given at weekly intervals. His physician, who referred the case, informs us that he had no further attacks.

CASE 2.—F. G., a Russian, widow, aged 32, gave a negative family history and had had no diseases or operations.

Four years before, the patient began to suffer from attacks of bronchial asthma, which had continued to the time of the beginning of treatment. The attacks recurred at intervals of a few days up to six weeks, and lasted from one to three days. During the attacks she had marked dyspnea, inability to cough or even speak, profuse perspiration and finally exhaustion. At the end of the

attacks the cough became more productive. She had never had hemoptysis, but had lost much weight.

The physical examination showed a woman of small build with the characteristic asthmatic type of chest. The heart was normal. The lungs showed scattered crepitations, but no wheezing râles at the time of the first examination. The auscultatory systolic blood pressure was 115 mm. of mercury, and the diastolic was 75 mm. (Tycos). The Wassermann test was negative. The urine was normal and the sputum, examined perhaps thirty times, showed no tubercle bacilli.

Ten subcutaneous injections of the patient's defibrinated blood were given at intervals of about one week, the amounts varying from 15 to 25 c.c. During the week after the first injection the patient felt better and stated that her previous intervals of relief had never been so complete. But the day after the second injection she had sneezing and cough which developed into an attack of considerable severity. The patient stated that no previous attacks were preceded by nasal symptoms or cough. The lungs showed prolonged expiration and scattered wheezing râles. The patient had a few more mild attacks, but after three injections they ceased and she began to gain in weight. The first injection was given Oct. 2, 1915, when she weighed 89 pounds. At the end of the treatment she weighed 92. Since then the patient has had no attacks of asthma, has gained about 9 pounds in three months, and but for a slight morning cough, states that she has felt remarkably well despite sudden changes of temperature and weather. Formerly she was unable to walk two blocks on account of dyspnea. She has lately been walking ten to fifteen blocks without any trouble twice daily, and has been working steadily.

Case 3.—A. O., a Russian, aged 35 years, gave a negative family history, and had previously been in excellent health except for occasional attacks of bronchitis. Three and a half years previously some nasal operation was performed. Two years before an appendectomy was done, since which time the present illness dates.

For two years the patient had suffered from spasmodic attacks of shortness of breath, wheezing in the chest, worse on exertion, moderate cough and expectoration, associated with a sensation of oppression in the epigastrium and lower half of the chest. These attacks occurred very frequently, averaging about four or five a week. The patient felt better indoors, but was not affected by walking in fields or woods.

Physical examination revealed a man weighing 218 pounds, with an emphysematous chest with an expansion of only 2.5 cm. between complete expiration and deep inspiration. The auscultatory systolic blood pressure was 140 mm. of mercury and the diastolic pressure was 90. The urine was normal and the Wassermann test was negative.

During the earlier period of his treatment we occasionally witnessed a typical paroxysm during which he was dyspneic and cyanotic, with his chest filled with wheezing and musical râles.

At intervals of about one week the patient received ten subcutaneous injections of defibrinated blood, according to the method above described. The amount varied from 15 to 22 c.c. The first injection was made Sept. 27, 1915, and the last on Dec. 12, 1915. After the first three injections the patient felt unimproved. He still had attacks of dyspnea and cough, with profuse expectoration and wheezing in his chest. Then the attacks ceased and have not reappeared since. In their stead he had for several weeks an almost constant cough with expectoration and fits of sneezing, soon succeeded by a severe frontal headache, which has somewhat lessened since.

At the time of the last injection and after that the patient stated that he felt much improved, in fact, that he had not felt so well at any time since the onset of his asthma. At one time he had a severe coughing spell which he feared might precede an attack, but none ensued. He has had no attacks in the past eighteen weeks following the termination of treatment.

His lungs at first showed, almost constantly, abundant wheezing râles. These have disappeared, though the expiration is still prolonged on account of the emphysema.

CASE 4.—G. F., a Hungarian, aged 30, gave a negative family and past history except for a hemorrhoid operation five years before.

The present illness dates back five years, when he commenced to have frequent colds and then typical attacks of bronchial asthma. He had dyspnea and a sense of oppression in the chest with cough and slight expectoration. A nasal operation performed two years before proved of no benefit. His attacks occurred weekly, but more often in winter. They were always aggravated by inclement weather, and seemed to bear no relation to the patient's diet.

Physical examination showed a typical asthmatic condition of the chest with diffuse wheezing and sonorous râles. The chest expansion was only 2 cm. The urine was normal. The Wassermann test was negative.

Treatment consisted of weekly subcutaneous injections of the patient's defibrinated blood, 15 to 25 c.c. each time, beginning Sept. 17, 1915.

For the first six weeks the patient stated that he felt practically well and had no attacks. He then had one mild attack and none after that until Feb. 8, 1916, when he had one severe attack. The patient stated, however, that for one year prior to the injections the attacks were almost constant. After the cessation of treatment the attacks were very mild and infrequent. The lungs still showed slight wheezing and prolonged expiration eight weeks after treatment was stopped.

CASE 5.—J. K., a Russian woman, aged 29, had a negative family and past history.

The present illness dates back many years (from the time she was three days old, she was told). She had attacks of dyspnea and wheezing in the chest of sudden onset, occurring about twice a week. There was sometimes an interval of a month between attacks, but rarely any longer.

The physical examination showed a congested throat and a slightly enlarged thyroid. The lungs showed a few piping basic râles. The chest expansion showed a variation of 4 cm. between deep expiration and deep inspiration. The urine was negative. The Wassermann test was negative.

The treatment consisted of subcutaneous injections of the patient's defibrinated blood at weekly intervals. About 15 c.c. were given each time. During the course of the treatment the patient had only two very mild attacks which occurred with severe changes of weather, and gained seventeen pounds in weight. Her lungs were clear with slightly prolonged expiration.

During the eight weeks following the last injection the patient had no attacks of asthma.

CASE 6.—P. F., a Russian woman, aged 29, gave a family history of asthma. In her past history there had been no illnesses or operations.

Four years previously, the patient began to cough, with profuse mucous expectoration. Then she was seized with attacks of dyspnea, which came rapidly and unannounced, with a sensation of oppression in the epigastrium and the front of the chest. She frequently had to sit at an open window and gasp for breath. Such attacks were repeated almost every night. During the five pregnancies which the patient went through she stated that she had practically no paroxysms. In the intervals between attacks she had only slight cough and substernal oppression. She would occasionally sneeze spasmodically eight to ten times during the day, and found that when the nasal symptoms predominated, the chest condition improved.

The physical examination revealed an emphysematous chest with slight wheezing over both lungs. The weight was 181 pounds. The urine was normal, and the Wassermann test was negative.

The treatment consisted of subcutaneous injections of the patient's defibrinated blood at intervals of about one week, the amounts given being 20 to 25 c.c. each time. The first injection was given Oct. 15, 1915.

Though after several injections the patient still had attacks, they were mild and infrequent while before the injections they were an occurrence of almost every night. In four months she had gained 2 pounds and stated that she felt the injections had helped greatly, and that during the treatment she was as well as four years before, when her condition was only incipient. After that she had a few mild attacks. Her weight at the termination of treatment was 189 pounds, a gain of eight pounds. The auscultatory systolic blood pressure was 115 mm. of mercury, and the diastolic pressure was 75 mm. (Tycos).

She became pregnant two months after the end of the treatment, and as she stated that pregnancy seemed a preventive of attacks of asthma in her case, the future course of her condition, if improved, may be attributed to that as much as to the treatment.

THEORETICAL CONSIDERATIONS

The process of reasoning by which we concluded that autogenous defibrinated blood might be of benefit in bronchial asthma may be summarized in the following outline, each step of which will be discussed later in detail:

1. Asthma is due to a spasm of the smaller bronchi.

2. Spasm of the bronchi in asthma is a manifestation of anaphylaxis.

3. The anaphylactic phenomena may be explained on the basis of protein sensitization.

4. Whether the protein gains access to the body by the nasopharynx, by the gastro-intestinal tract, by the respiratory system or by some other portal, it is probably absorbed by the blood; if so, it should be found in the blood, especially just prior to or during an asthmatic attack.

5. The rational method of active immunization in anaphylaxis consists of repeated injections of small doses of the causal protein; if the previous premises are true in asthma, immunization by repeated parenteral injections of autogenous defibrinated blood, obtained preferably during a paroxysm, should be beneficial.

ASTHMA A RESULT OF SPASM OF THE BRONCHIOLES

That the attacks of asthma are the result of muscular spasm of the smaller bronchi was originally stated by Reisseisen[1] and has been most ably contended by Hyde Salter[2] as far back as 1858. The excellent work of Beer,[3] Riegel,[4] Lazarus[5] and others has gone far to confirm this hypothesis. The experiments of Brodie and Dixon[6] have clearly

1. Reisseisen: System of Medicine, Allbutt and Rolleston, London, 1909, v, 51.
2. Salter: On Asthma, Its Pathology and Treatment, New York, 1882.
3. Beer: Arch. f. Anat. u. Physiol., 1892, Supplement, p. 101.
4. Riegel and Edinger: Ztschr. f. klin. Med., 1882, v, 413.
5. Lazarus: Deutsch. med. Wchnschr., 1891, xvii, 852.
6. Brodie and Dixon: Tr. Path. Soc. London, 1903, liv, 17; Jour. Physiol., 1903, xxix, 97.

demonstrated the typical relationship between bronchial spasm and the syndrome known as bronchial asthma. Stimulation of the constrictor fibers of the vagus by muscarin or pilocarpin or by electricity invariably results in the production of the signs and symptoms of bronchial asthma. Further evidence favoring this theory is afforded by the sudden onset and almost equally as sudden a cessation of the asthmatic attack—the usual occurrence in spasm of any involuntary muscle.

The hypothesis next in order of importance is that which assumes a rapid turgescence of the mucous membrane of the bronchioles. This seems to be less tenable and is being discarded by many. The various other theories as to the causation of bronchial asthma need not be discussed in this paper.

ASTHMA AND ANAPHYLAXIS

Studies in anaphylaxis have of late years served to make clearer the conception of the pathogenesis of asthma. Richet and Hericourt in 1898 applied the name anaphylaxis to the syndrome which results from the injection of a soluble protein into an animal sensitized to it.[7] This syndrome consists of marked respiratory distress, analogous to an asthmatic paroxysm, cyanosis, vomiting, and asphyxia, which may prove fatal.

The work of Meltzer[8] and the experimental studies of Auer and Lewis tend to prove that the asthmatic individual is sensitized to a definite substance, and that an asthmatic attack sets in every time this substance happens in some way to enter into the circulation of the individual. In the actual processes which take place in nature the effective doses may be infinitely small. It may well be possible that the minute quantities contained in the emanation from horses, cats or guinea-pigs are sufficient to act as a toxic dose, and to call forth the nonfatal stenosis of the bronchi evidently present in an asthmatic attack. Auer and Lewis' investigations seem definitely to establish acute anaphylactic death as being caused by respiratory failure consequent on stenosis of the bronchi.[9] The resulting asphyxia is due to an inspiratory immobilization of the lungs.

Gillette collected, up to 1909, fifteen fatal cases and thirteen nonfatal cases of severe anaphylaxis following injections of horse serum.[10] Of the total twenty-eight cases, eighteen were in asthmatics, of whom nine died. Inasmuch as these reactions were due to horse serum, it is noteworthy that in a number of these individuals previous asthmatic attacks were incited by being near horses.

7. Richet: Compt. rend. Soc. de biol., 1898, v, 137.
8. Meltzer: Jour. Am. Med. Assn., 1910, lv, 1022.
9. Auer and Lewis: Jour. Am. Med. Assn., 1909, liii, 458.
10. Gillette: Therap. Gaz., 1909, xxxiii, 159.

ANAPHYLAXIS AND PROTEIN SENSITIZATION

When a foreign protein gains entrance into the body, it has been shown that certain cells develop a specific digestive ferment which splits up the invading protein. The resulting split products, according to Vaughan,[11] possess the property of sensitizing the individual to the unbroken molecule. The first or sensitizing dose of protein injected into an animal is digested, but the process goes on so slowly that no toxic effect is evident. An immediate effect on reinjection depends on the rapidity with which the protein is split up and its poisonous constituent set free.

Certain digestion products of the proteins may now and then become absorbed in an abnormal stage into the circulation from the alimentary canal and produce an asthmatic attack. Babcock considers asthma an anaphylaxis due to the absorption of bacterial protein from some focus of infection.[12] He believes the focus is most frequently located in the respiratory tract itself, but may be in the gallbladder or in other parts of the body.

Though poisonous molecules may be bacterial, vegetable or animal in origin, they are not distinguishable in physiologic effect, all having the property of exciting the anaphylactic attack.

THE PROTEIN IN THE CIRCULATION

If we assume then that asthma is an anaphylactic reaction due to the introduction into the system of a foreign protein, the next step is to attempt to identify this protein, or if that is not possible, to discover its source, portal of entry and localization. Comparatively little work has been done along this line. We are now attempting some researches in this connection which may prove of some value and which will be reported in a future publication.

In its analogous malady, hay-fever, Koessler attempted to demonstrate the presence of pollen protein free in the circulating blood of patients suffering with a seasonal attack.[13] He took blood from a patient during an attack of hay asthma. Five c.c. of serum were injected subcutaneously into each of four guinea-pigs. Twelve to eighteen days later an intracardial injection was made of 1 to 10,000 dilution of an extract of ragweed pollen, and three out of the four guinea-pigs showed symptoms of anaphylaxis. The deduction that Koessler made from his experiment was that there was a pollen protein in the serum of his patients. Friedberger[14] has shown that the serum

11. Vaughan: Am. Jour. Med. Sc., 1913, cxlv, 161; Protein Split Products, New York, 1913.
12. Babcock: Jour. Am. Med. Assn., 1915, lxv, 1942.
13. Koessler: Illinois Med. Jour., 1914, xxvi, 120.
14. Friedberger: Ztschr. f. Immunitätsforsch. u. exper. Therap., 1910, iv, 636.

of rabbits treated with a large dose of sheep serum is capable by itself, when injected intravenously, of producing anaphylactic shock in guinea-pigs sensitized to sheep serum. From this he argues that a small amount of sheep serum remained uneliminated from the rabbits' circulation.

Weil has shown that the biologic reaction between antigen and antibody takes place in the cells of the body, such as involuntary muscle, and not in the circulating blood;[15] the assumption is that the antigen reaches the cells through the circulation and may there, in transit, be discovered.

Similarly Schloss[16] believes that the occurrence of an urticarial lesion from cutaneous inoculation of toxic foods is directly comparable to the more striking clinical effects produced by their ingestion, inasmuch as they must in the latter case necessarily reach the skin through the circulation.

The assumption seems therefore warranted that just prior to the occurrence of an anaphylactic attack, and clinically at the time of onset and probably also during an attack of asthma, the toxic protein which excites the attack is free in the circulating blood.

IMMUNIZATION IN ANAPHYLAXIS AND ASTHMA

Active immunization to the toxic protein in anaphylaxis may be obtained by injecting the protein in small amounts subcutaneously in successive doses at short enough intervals and over a long enough period of time. Schloss succeeded in immunizing five cases of egg-sensitization by feeding small doses of egg-albumin. In one case of urticaria, treatment was begun by the administration of 2 mg. of ovomucoid in capsules three times daily. The dose was increased at first gradually and then more rapidly. The progress of immunization was determined by means of the cutaneous reaction. The latter finally became negative and the patient was completely immunized to egg or ovomucoid.

Goodale has recorded the case of a patient with horse asthma with a high initial sensitization to horse serum, who acquired an evident increase in resistance in the course of thirty parenteral injections of progressively increasing doses of horse serum.[17] He determined the increase in resistance by the application of horse serum to an abrasion of the skin, which in sensitized individuals produces a sharply localized edema and reddening within a few minutes. Levison[18] treated two

15. Weil: Jour. Med. Research, 1914, xxx, 87; ibid., 1915, xxxii, 107.
16. Schloss: Am. Jour. Dis. Child., 1912, iii, 341.
17. Goodale: Boston Med. and Surg. Jour., 1915, clxxii, 751; ibid., 1915, clxxiii, 42.
18. Levison: New York Med. Jour., 1915, cii, 901.

patients with horse asthma in the same way with similar beneficial results.

Babcock, assuming the existence of an infectious focus in the body from which the foreign protein is absorbed, considers autogenous vaccines the logical procedure in the treatment of asthma. In 1913 Duncan[19] attempted to immunize a patient suffering with asthma by repeated injections of filtered toxins from the patient's sputum.

Rosenau and Amoss[20] asserted that the condensed expired air from human beings contains a substance which sensitizes guinea-pigs to human blood serum. One of us (Kahn),[21] in collaboration with Weisman, made observations on the effect on asthma of the injection of liquid from human expired air. The injection was made into the median basilic vein of two persons, one who was normal receiving 5 c.c., and another, aged 30, who had been a sufferer with bronchial asthma, receiving 8 c.c., during an interval between attacks. There were no immediate or after effects noted in either case. The further work of Weisman definitely disproved the statements of Rosenau and Amoss that the breath contains a "volatile" protein, and that such "volatile" protein is an important respiratory factor.

Holbrook Curtis,[22] Dunbar,[23] Noon and Freeman,[24] Clowes[25] and Koessler have used the method of active immunization in hay-fever. More recently Oppenheimer and Gottlieb[26] have reported favorably on the injection of very small amounts of pollen extract at about weekly intervals just previous to or during a seasonal attack. They used the cutaneous test to discover the specific causal pollens. Hays[27] reported twelve patients with hay-fever most of whom received two subcutaneous injections of from 5 to 10 c.c. of autogenous serum. He reported negative results.

COMMENT

In bronchial asthma there seems to exist a theoretical foundation for this method of treatment. With each attack of asthma there occurs a sudden or gradual liberation into the circulating blood of the protein which excites the attack. If then, during that period blood is withdrawn, it presumably contains the toxic protein or antigen. This can now be considered equivalent to the isolation of the substance which is to be used for active immunization, regardless of its source of origin. Repeated injections of this defibrinated blood subcutaneously are made

19. Duncan: New York Med. Jour., 1913, xcvi, 1278.
20. Rosenau and Amoss: Jour. Exper. Med., 1913, xvii, 132.
21. Weisman: Biochemical Studies of Expired Air in Relation to Ventilation, Diss., Columbia Univ., New York, 1913.
22. Curtis: New York Med. News, 1900, lxxvii, 16.
23. Dunbar: Deutsch. med. Wchnschr., 1911, xxxvii, 578.
24. Noon and Freeman: Lancet, London, 1911, i, 1572.
25. Clowes: Soc. for Exp. Biol. and Med., 1913, x, 48.
26. Oppenheimer and Gottlieb: New York Med. Jour., 1915, ci, 229.
27. Hays: Ann. Otol., Rhin. and Laryng., 1915, xxiv, 285.

at varying intervals. The quantities of antigen contained in each injection are so minute as to create a very small amount of antibody each time, and never to result in an attack. Thus the tolerance of the body for the protein may be enhanced.

Naturally in an ideal course of treatment it would be desirable to obtain the blood each time just prior to or early during an attack of asthma, since we assume that it is then that the antigen is in the circulation in the greatest amount.

It is possible that the benefit from the treatment may rest on an empirical basis. The objection may be raised that the amount of antigen in circulation is greater than we can withdraw for reinjection. Especially can it be contended that the mere withdrawal of blood from the circulation and its reintroduction subcutaneously is not sufficient to produce a marked biologic effect on the involuntary muscles. It may also be said that the original presence of antigen in the circulation should itself serve to immunize the individual. But this, we know, does not take place in asthma, whereas the clinical results from the treatment would seem to permit of our foregoing interpretation.

It is obvious that the cases reported are too few from which to draw any definite conclusions as to the efficiency of autotherapy with defibrinated blood. But the results in these cases are promising enough to justify a continuation of the treatment and to find in what proportion of the cases it is of benefit, and to what degree.

In a disease in which the physical signs are so transitory as they are during the attacks of bronchial asthma, improvement or amelioration should not be judged merely by the changes in physical signs. The patient's subjective sensations should bear at least equal if not greater weight in forming our opinion of betterment.

CONCLUSIONS

1. Inasmuch as bronchial asthma is due, in our opinion, to sensitization by a foreign protein, immunization by injection of the causal protein should be of benefit.

2. Autogenous defibrinated blood, obtained preferably during the attacks of asthma, we assume may serve as antigen for treatment by active immunization.

3. Six successive patients with bronchial asthma, treated by repeated injections of autogenous defibrinated blood, have shown definite improvement as indicated by diminution in frequency and severity of attacks, gain in weight, increased ability to work and improved subjective symptoms.

We wish to thank Dr. Isidor N. Kahn and Dr. N. Ereiter for their kind interest in this work and for referring several of the cases to us for treatment.

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THE ALBUMIN AND GLOBULIN CONTENT OF HUMAN BLOOD SERUM

IN HEALTH, SYPHILIS, PNEUMONIA, AND CERTAIN OTHER INFECTIONS, WITH THE BEARING OF GLOBULIN ON THE WASSERMANN REACTION *

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In spite of numerous results found in literature, the albumin and globulin content of serum in health and disease has been reinvestigated by use of Robertson's[1] method, because of the simplicity and freedom from possible error of the new technic as compared with that of former methods. The small amount of serum used is another advantage and has made possible the use of two controls on each serum examined by the writer. The results which can be obtained are uniform, especially if the writer's suggestions about the technic, which will soon be published, are followed and his automatic pipet[2] is used to assure accuracy of measurements.

During the last seven months a number of normal serums have been examined. The average results differ slightly from those obtained by Robertson's method previously reported.[3] The total protein is lower than in the previous series, due to the fact that serums were taken, except in a few cases, from patients who were confined to bed as a result of a fracture or uncomplicated herniotomy, or from those who had been lying down for twenty-five or more minutes. The non-proteins are slightly higher, while the percentage of globulin in the total protein is moderately increased. The last four serums were taken from the same person at different times throughout a period of six months. The values are quite uniform (Table 3).

LITERATURE

The literature contains many results for the normal value of serum proteins, most of which will be found in Table 1. Several of the average values were taken directly from the articles by Erben.[4] Com-

* Submitted for publication June 13, 1916.

* From the Medical Service and Chemical Laboratory of the Massachusetts General Hospital.

1. Robertson, T. B.: Jour. Biol. Chem., 1915, xxii, 233.
2. Rowe, A. H.: Jour. Lab. and Clin. Med., 1916, i, 439.
3. Tranter and Rowe: Jour. Am. Med. Assn., 1915, lxv, 1433.
4. Erben, F.: Zetchr. f. klin. Med., 1903, l, 450; ibid., 1900, xl, 266, 282; Ibid., 1902, xlvii, 302; Ztschr. f. Heilk., 1905, xxvi, 245, 303, 449.

TABLE 1.—NORMAL VALUE OF SERUM PROTEINS AS FOUND IN THE LITERATURE

Investigator	Where Found	Date	Percentage of Total Protein
Berzelius	Erben: Ztschr. f. klin. Med., 1903, l, 450; ibid., 1900, xl, 266, 282; ibid., 1912, xlvii, 302; Ztschr. f. Heilk., 1905, xxvi, 245, 303, 449	1831	8
Marcett	Erben: Ztschr. f. klin. Med., 1903, l, 450; ibid., 1900, xl, 266, 282; ibid., 1912, xlvii, 302; Ztschr. f. Heilk., 1905, xxvi, 245, 303, 449	1831	8.7
Denis	Erben: Ztschr. f. klin. Med., 1903, l, 450; ibid., 1900, xl, 266, 282; ibid., 1912, xlvii, 302; Ztschr. f. Heilk., 1905, xxvi, 245, 303, 449	1838	8
Lecanu	Erben: Ztschr. f. klin. Med., 1903, l, 450; ibid., 1900, xl, 266, 282; ibid., 1912, xlvii, 302; Ztschr. f. Heilk., 1905, xxvi, 245, 303, 449	1837	7.8 to 8.1
Bostok	Erben: Ztschr. f. klin. Med., 1903, l, 450; ibid., 1900, xl, 266, 282; ibid., 1912, xlvii, 302; Ztschr. f. Heilk., 1905, xxvi, 245, 303, 449	1842	10
Nasse	Erben: Ztschr. f. klin. Med., 1903, l, 450; ibid., 1900, xl, 266, 282; ibid., 1912, xlvii, 302; Ztschr. f. Heilk., 1905, xxvi, 245, 303, 449	1836	7.2 to 9
Becquerel and Rodier	Gaz. méd. de Paris, 1844, Nos. 47, 48, 49, 50, 51; Chemie pathologique, 1854; Untersuchungen über de Zusammensetzung des Blutes in gesunden und Kranken Zustande, übersetz von Eisenmann, Arlangen, 1845	1845	8
Otto	Erben: Ztschr. f. klin. Med., 1903, l, 450; ibid., 1900, xl, 266, 282; ibid., 1912, xlvii, 302; Ztschr. f. Heilk., 1905, xxvi, 245, 303, 449	1848	7.5 to 8
C. Schmidt	Charakteristik der epidem. Cholera, Leipzig, 1850, p. 30; reference from Joachim	1850	7.4 to 8.3
Leven	Malay's Jaresbericht, 1873, i, 115	1873	7.9
Hammarsten	Arch. f. d. ges. Physiol., 1878, xvii, 413; Ergebn. d. Physiol., 1902, i, 348; Lehrbuch der physiologischen Chemie, 1914	1878	7 to 8.1
Hoffman	Arch. f. exper. Path., 1882, xvi, 133	1882	7.4 to 7.8
Limbeck and Pick	Prag. med. Wchnschr., 1893, xviii, 21, 133, 149, 165; Deutsch. med. Wchnschr., xx, 563. Limbeck: Grundriss einer klin. Path. d. Blutes, Ed. 2., Jena, S. Fischer, 1896, p. 100	1893	6.5 to 7.4
Von Jaksch	Ztschr. f. klin. Med., 1893, xxiii, 187	1893	6 to 8.9
Lewinsky	Arch. f. d. ges. Physiol., 1905, c, 611	1905	6.7 to 7.6
Erben	Erben: Ztschr. f. klin. Med., 1903, l, 450; ibid., 1900, xl, 266, 282; ibid., 1912, xlvii, 302; Ztschr. f. Heilk., 1905, xxvi, 245, 303, 449	1905	8.5
Refractometric determinations by Reiss and others	Ergbn. d. inn. Med. u. Kinderh., 1913, x, 531	1902 1912	7 to 9
Winternitz	Arch. f. Dermat. u. Syph., 1908, xciii, 65; ibid., 1910, cl, 227	1908	8.2 to 8.7
Epstein	Jour. Exper. Med., 1912, xvi, 719; ibid., 1913, xvii, 444; ibid., 1914, xx, 234	1912 1913	6.4 to 8.3
Tranter and Rowe	Jour. Am. Med. Assn., 1915, lxv, 1433	1915	6.7 to 8.7
Rowe	This article	1916	6.5 to 8.2

ment on these values from the literature will be found in the last portion of this review of the literature.

The best reviews of the literature on serum proteins in health and disease occur in the articles by Limbeck and Pick,[5] Joachim,[6] Von Jaksch,[7] Langstein and Mayer,[8] Erben,[4] and Winternitz.[9] Morawitz[10] discusses the methods for determining albumin and globulin and the results obtained up to 1908 in human serum, concluding that little of importance to pathology and physiology has come from the extensive work in this field. This opinion is due, probably, to the disparity of many of the former results.

The monograph of Becquerel and Rodier[11] is remarkable considering the early date of its production. It covers the chemistry of the blood in health and disease in a very thorough manner. In chlorosis and plethora it was interesting to note that the total proteins were normal, while in nephritis and cardiac disease with edema, endocarditis, typhoid, acute rheumatic fever, pneumonia, secondary anemias and pulmonary tuberculosis they were decreased.

C. Schmidt[12] found serum proteins increased in severe cholera and decreased in nephritis.

Hoppe-Seyler[13] found the serum proteins decreased in a woman with chyluria.

Leven[14] obtained 7.67 per cent. of protein in the blood serum from a patient with scorbutus.

Estelle[15] determined the albumin and globulin content in the blood serum of two nephritics, in one the albumin being 5.44 per cent. and the globulin 3.06 per cent., while in the other the values were 3.6 and 1.8 per cent., respectively.

Csatary[16] found increased serum globulin in nephritis.

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5. Limbeck and Pick: Prag med. Wchnschr., 1893, xviii, 21, 133, 149, 165; Deutsch. med Wchnschr., 1894, xx, 563. Limbeck: Grundriss einer klin. Path. d. Blutes, Ed. 2, Jena, S. Fischer, 1896, p. 100.

6. Joachim: Arch. f. d. ges. Physiol., 1893, 588; Wien. klin. Wchnschr., 1902, xv, 565.

7. Von Jaksch: Ztschr. f. klin. med., 1893, xxiii, 187.

8. Langstein and Mayer: Beit. z. chem. Phys. u. Path., 1904, v, 69.

9. Winternitz, R.: Arch. f. Dermat. u. Syph., 1908, xciii, 65; ibid., 1910, ci, 227.

10. Morawitz: Blood Proteins, Oppenheimer's Handbuch der Biochemie des Menschen und der Tiere, 1909, ii, Part 2, 70.

11. Becquerel and Rodier: Gaz. méd. de Paris, 1844, Nos. 47, 48, 49, 50, 51; Chemie pathologique, 1854; Untersuchungen über die Zusammensetzung des Blutes in gesunden und kranken Zustande, übersetz von Eisenmann, Erlangen, 1845.

12. Schmidt, C.: Charakteristik der epidem. Cholera, Leipzig, 1850, p. 30, reference from Joachim.

13. Hoppe-Seyler: Ztschr. f. physiol. Chem., xv, 179.

14. Leven, M.: Maly's Jahresbericht, 1873, i, 115.

15. Estelle: Revue mens. de méd. et de chir., 1880, iv, 704.

16. Csatary: Arch. f. klin. Med., 1891, xlviii, 358.

Nya-Viglezio[17] found increased globulin in pneumonia, angina pectoris, tetanus, nephritis, diabetes, the values varying from 4.8 to 3.6 per cent. for albumin and from 3.5 to 3 per cent for globulin.

Von Jaksch,[7] using serum obtained by cupping, showed a dilution of blood in acute illnesses and diseases of the heart, lungs, and kidneys. In leukemia he found 7.6 per cent protein, in pernicious anemia 7.4 per cent., in chlorosis 8.25 per cent., while in secondary anemias the protein was slightly reduced.

TABLE 2.—NORMAL VALUES FROM LITERATURE FOR ALBUMIN AND GLOBULIN WITH THE PERCENTAGE OF GLOBULIN IN THE TOTAL PROTEIN

Investigator	Where Found	Comment	Albumin	Globulin	Percentage of Globulin
Hammarsten..	Arch. f. d. ges. Physiol., 1878, xvii, 413; Ergebn. d. Physiol., 1902, i, 348; Lehrbuch der physiologischen Chemie, 1914	Average values of 4 sera obtained by cupping*	4.34	3.22	43
Patein.....	Jour. pharm. Chemie, 1899, x, 249.....	.....	4.89	2.86	37
		.....	4.63	2.77	37
Nya-Viglezio...	Archivio italiano di clinica medica, 1888, xxvii; reference from Joachim; Revista Clinica, 1887, xxvi, 673	.....	5.72	2.43	30
Limbeck and Pick	Prag. med. Wchnschr., 1893, xviii, 21, 133, 149, 165; Deutsch. med. Wchnschr., 1894, xx, 563. Limbeck: Grundriss einte klin. Path. d. Blutes, 1896, 100	Nervous diseases (six cases)	4.8	1.7	26
Lewinski.....	Arch. f. d. ges. Physiol., 1902, c, 611.....	.....	3.9	2.96	43
Winternitz.....	Arch. f. Dermat. u. Syph., 1908, xciii, 65; ibid., 1910, ci, 227	.....	6.2	2	24
Epstein.....	Jour. Exper. Med., 1912, xvi, 719; ibid., 1913, xvii, 444; ibid., 1914, xx, 334	Average values of 7 cases (varicocele, orchitis, fistula, hemorrhoids)	4.4	2.7	38
Epstein.....	.....	One normal case.....	5.00	3.07	38
Hoffmann.....	Arch. f. exper. Path., 1882, xvi, 133.....	.....	5.04	2.72	35

* All other values obtained from venous blood serum.

Limbeck and Pick[8] showed an increased globulin content in the serum of nineteen cases of acute infection, though the total proteins were much reduced. Five diabetics showed increased globulin, while three cases gave low globulin. In nephritis thirteen cases showed a low total protein. The results of these writers were undoubtedly too low and were criticized by Bleibtreu.[18] The latter was at fault, though, in using as his normal values those obtained from blood taken post mortem.

17. Nya-Viglezio: Archivio italiano di clinica medica, 1888, xxvii; reference from Joachim, Revista clinica, 1887, xxvi, 673.
18. Bleibtreu: Deutsch. med. Wchnschr., 1893, xix, 1167.

E. Freund[19] found that the percentage of globulin in nephritis varied from 25 to 33 per cent., while in pernicious anemia it was 33 per cent.

Emmerich and Tsuboi[20] stated that the globulin in blood serum decreased to zero in animals immune to hog cholera and pneumonia. This statement was never confirmed.

Seng[21] found increased globulin in serum from animals immune to diphtheria.

Atkinson[22] showed that the antitoxic power of a serum was caused by the globulins.

V. Szontagh and Wellmann[23] found that with immunization the total proteins were slightly increased.

Butjagen[24] confirmed the former findings of V. Szontagh.

Joachim[6] in one article studied the protein of the blood serum, showing that globulin increased in many diseased conditions In a second article the globulin content of ascitic fluids was determined and he also demonstrated that globulin increased at the cost of albumin when a horse was immunized to diphtheria, the albumin before immunization being 5 per cent. and afterwards dropping to 3.7 per cent.

Jakoby[25] found euglobulin increased in animals immunized to castor oil.

Moll[26] found in animals injected with various proteins, gelatin, and killed bacteria a marked increase in the globulin content and concluded that with an increase in immune bodies there goes an increase in globulin. He suggested that possibly some globulin arises from leukocytes, which suggestion first came from A. Schmidt.[27]

Glaessner[28] repeated the above work, showing a moderate increase in the globulins, but not to the extent found by Moll. He could demonstrate no constant relation between the globulin and immune body formation. He suggested that the globulin has a greater resistance to the proteolytic ferment trypsin than has albumin, to account for its relative increase in disease.

Langstein and Mayer[5] found the serum proteins increased in immunized and infected rabbits, the increase being largely in the glob-

19. Freund, E.: Wien. klin. Rundschau, 1895, ix, 49.
20. Emmerich and Tsuboi: Verhandl. d. Cong. f. inn. Med., 1892, ii, 202.
21. Seng: Ztschr. f. Hyg. u. Infekt., 1899, xxxi, 513.
22. Atkinson: Jour. Exper. Med., 1900, v, 67.
23. V. Szontagh and Wellmann: Deutsch. Med. Wchnschr., 1898, xxiv, 421.
24. Butjagen: Hyg. Rundschau, 1902, xii, 1193.
25. Jakoby: Beitr. z. chem. Phys. u. Path., i, 59.
26. Moll: Beit. z. chem. Phys. u. Path., 1903, iv, 563, 578.
27. Schmidt, A.: Zur Blutlehre, Leipzig, 1892. Beiträger zur Blutlehre Wiesbaden, 1895. Ref. from Hammarsten.
28. Glaessner: Ztschr. f. exper. Path. u. Therap., 1905, ii, 154.

ulin fraction. They were unable to confirm the work of Emmerich and Tsuboi.

Müller[29] confirmed the findings of Langstein and Mayer.

TABLE 3.—Albumin and Globulin Content of Human Blood Serum in Normal Cases

Case No.	Age	Albumin	Globulin	Total Protein	Nonprotein	Percentage Globulin
1	27*	5.85	1.97	7.82	1.2	25
2	30	5.15	2.2	7.35	1.3	30
3	30*	5	2.3	7.3	1.3	32
4	21*	5.7	2	7.7	1.3	26
5	24*	6.4	1.2	7.6	1.1	16
6	30	5	2.4	7.4	1.2	32
7	32	5.8	2.2	8	1.1	27.5
8	48	5.8	2.1	7.9	1.2	27
9	19	6	2.2	8.2	1.2	27
10	25	5.7	2	7.7	1.3	26
11	48	5.1	2.2	7.3	1.2	30
12	28	4.8	2	6.8	1.3	29
13	23	5.6	1.8	7.4	1.2	24
14	19	4.6	1.9	6.5	1.25	29
15	48	4.6	2.1	6.7	1.25	31
16	25	5.9	1.6	7.5	1.3	21
17	26	5	1.7	6.7	1.3	25
18	29	5.4	1.4	6.8	1.3	21
19	3/18/16 26 1/26/16*	6	1.5	7.5	1.3	20
20	26 10/19/16*	6.5	1.7	8.2	1.3	21
21	26 10/11/16*	6.7	1.5	8.2	1.3	18
22	26	6	1.9	7.9	1.1	24
Averages.....		5.6	1.9	7.5	1.24	25.5
Former averages.....		6.2	1.74	7.94	1.1	22

* These subjects had been walking about without doing any real work before blood was taken.

Lewinski[30] found the percentage of globulin a little higher in pregnant and a little lower in eclamptic women than in normal ones.

In a case of pernicious anemia Erben[4] found 5.2 per cent. total protein, of which 4.2 per cent. was albumin and 1 per cent. globulin.

29. Müller, Paul: Beitr. z. chem. Phys. u. Path., 1905, vi, 454.
30. Lewinski: Arch. f. d. ges. Physiol., 1903, c, 611.

In parenchymatous nephritis the albumin was reduced to below 1 per cent., but was not so reduced in amyloid. The globulin was slightly increased in pericarditis. In tuberculosis the globulin was moderately increased as compared with normal values of Erben. He offers the suggestion of Gottwalt[31] that the relative increase of globulin in infections is due to its greater resistance to the toxins. In a case of carcinoma a slight reduction in total protein of the serum occurred though the relation of albumin to globulin was normal. In diabetes the total protein was either slightly decreased or increased, which confirmed the findings in previous literature with the exception of those of Limbeck and Pick. In a case of chronic lead poisoning no definite changes in the serum protein occurred. In a case of typhoid no values for albumin and globulin were given.

The articles of Erben include extensive bibliographies on the entire chemistry of the blood. Normal values of all organic and inorganic substances investigated in the blood are given, together with a table of the average normal values from the literature before 1905.

Vadala,[32] in two cases of anemia from ankylostomiasis, obtained the average value of 7.8 per cent. for serum albumin and 2.4 per cent. for globulin.

Grenet,[33] Gilbert and Chiray[34] found serum proteins decreased in liver insufficiency with and without ascites.

Winternitz[9] found serum globulin moderately increased in syphilis.

Epstein[35] obtained a marked increase in globulin with a normal or very subnormal amount of total serum protein in cardiac diseases associated with decompensation and serous effusions, pulmonary and respiratory affections of inflammatory or noninflammatory origin (pneumonia, emphysema, polycythemia), diabetes mellitus and parenchymatous nephritis. Globulin was normal or decreased in achylia gastrica, tuberculosis, diabetes insipidus and chronic interstitial nephritis. In another paper he found localized infections of the kidney gave an increase in globulin, while the cases of prostatic hypertrophy and minor surgical cases gave no such increase.

The methods for determination of albumin and globulin in the past may be summarized as follows: (1) by diluting the serum ten times, precipitating out the globulin by adding salt, or obtaining the globulin by dialysis; (2) by precipitating the globulin by acetic or carbonic acid; (3) by salting out the globulin by neutral salts: (a) Ham-

31. Gottwalt: (Reference given by Limbeck incorrect.) Correct reference, Ztschr. f. phys. Chem., 1880, iv, 423, 427.
32. Vadala: Clinica med. ital., 1907, vi; reference from Biol. Centralbl., 1907, vi, No. 2480.
33. Grenet, H.: Compt. rend. Soc. de. biol., 1907, lxiii, 552.
34. Gilbert and Chiray: Compt. rend. Soc. de biol., 1907, lxiii, 487.
35. Epstein: Jour. Exper. Med., 1912, xvi, 719; ibid., 1913, xvii, 444; ibid., 1914, xx, 334.

mersten's method utilizes magnesium sulphate after which the precipitate is washed, dried and weighed, or the nitrogen in the precipitate is estimated by the Kjeldahl[36] method and the protein obtained by multi-

TABLE 4.—ALBUMIN AND GLOBULIN CONTENT OF HUMAN BLOOD SERUM IN CASES OF SYPHILIS *

Case	Clinical Diagnosis	Wassermann	Treatment	Serum Examination				
				Albumin	Globulin	Total Protein	Nonprotein	Globulin %
1	Chancroid	Negative	None	6.4	1.2	7.6	1.1	16
2	Early secondary	Spirochetes demonstrated	None	5.2	2.3	7.6	1.1	30
3	Early malignant	+++	None	4.6	3.3	7.9	1.35	42
4	Early secondary	+++	None	5.4	2	7.4	1.3	27
5	Early secondary	+++	None	6.1	2.2	8.3	1.35	27
6	Tertiary	+++	Irregular for 2 yrs.	4.8	2.6	7.4	1.2	35
7	Early secondary	+++	2 Hg injections	5.8	2.2	8	1.3	27.5
8	Early secondary	+++	None	3.7	2.6	7.3	1.2	49
9	Tertiary	+++	2 doses salv.	4.7	2.6	7.3	1.25	36
10	Early secondary	+++	1 dose salv. and Hg pills	4.2	2.7	6.9	1.2	40
11	Tertiary	++	1 dose	4.6	2	6.6	1.2	30
12	Gastric ulcer (no evidence of lues)	Faintly +	None	5.7	1.2	6.9	1.1	17
13	Tertiary aneurism	+++	None	4.6	2.5	7.1	1.5	35
14	Secondary	+++	None	5.2	2.9	8.1	1.3	36
15	Late—6 yrs.	+	None	4.5	2.9	7.4	1.4	39
16	Early secondary	+++	1 dose salv. and Hg pills	5.5	2.4	7.9	1.4	30
17	Secondary	+++	Hg pills for 1 week	5.3	2.6	7.9	1.3	33
18	Late secondary	+++	Slight	5.4	1.9	7.3	1.4	26
19	Late secondary	+++	Salv. and Hg	5.6	2.5	8.1	1.4	31
Averages.....				5	2.5	7.5	1.3	33.7

* Cases 1 and 12 were not used to obtain average values. All but three of these cases came from the South Medical department. Dr. C. Morton Smith furnished the diagnosis.

plying the nitrogen content by 6.25, as advised by König and Kisch.[37] The latter technic was described by Hoppe-Seyler.[38] (b) Hofmeister,[39] Pohl,[40] Kauder,[41] and Reye[42] used ammonium sulphate. Otherwise the method is the same as the former one.

36. Kjeldahl: Ztschr. f. anal. Chem., 1883, xxii, 366.
37. König and Kisch: Ztschr. f. anal. Chem., 1889, xxviii, 193.
38. Hoppe-Seyler: Handbuch der phys. u. path. chem. Analyse, 1893.
39. Hofmeister: Ztschr. f. phys. Chem., xx, 319.
40. Pohl: Arch. f. exper. Path. u. Pharmakol., 1886, xx, 426.
41. Kauder: Arch. f. exper. Path. u. Pharmakol., 1886, xx, 411.
42. Reye: Inaug. Diss., Strassburg, 1898.

There has been great divergence of opinion about the accuracy of these methods, well explained by Nellis Foster.[43] Howell[44] states that the precipitate of globulin by ammonium sulphate is unsatisfactory because of the changed properties of the globulin after precipitation. Robertson's method, in utilizing ammonium sulphate, thus carries with it some unavoidable objections. Still these objections are the same in all instances and thus deductions can safely be drawn from values obtained from pathologic cases. But in doing away with the evident sources of error arising from the complicated technic of the older methods and in utilizing small amounts of blood, Robertson's method is undoubtedly the most satisfactory one yet suggested. An experience of one year with the technic has shown me that uniform results are obtained, which, though they are much lower in globulin than former results, are thought to be dependable. The cause of this difference from former results will be investigated later.

The results given in literature for the total proteins have been more uniform than those for albumin and globulin. The refractometer was introduced by Strubell in 1900 to determine quantitatively the serum proteins, the method being perfected by Reiss in 1902. Since then many data dealing with total protein in human serum have been added to literature, the chief workers having been Achard, Ascher, Bohme, Chiray, Chajes, Engel, Koranyi, Bence, Luthje, Oppenheimer, Schwenker, Strauss, Widal and Vaucher. References to all this work done with the refractometer are given in the comprehensive article recently published by Reiss.[45] The results are in most points like those arrived at by former methods.

In 1913 Schorer[46] studied Reiss' refractometric method and concluded that the changed relation between albumin and globulin in disease gives rise to a definite error in estimating the total protein. This was because the refractive index of albumin is less than that of globulin as determined by Reiss, Robertson, and confirmed by Schorer. That the total protein as estimated by the Kjeldahl-Kolben method, or by precipitation by alcohol and weighing, agreed within a few tenths of 1 per cent. with the refractometric estimation was the result of work done by Reiss,[47] Widal and Laudat, and Tuffier and Maute.[48] Chiray and Démanche[49] and also Schorer[50] had been unable to obtain such

43. Foster, N.: The Archives Int. Med., 1912, x, 415.
44. Howell: Am. Jour. Physiol., 1906, xvii, 280.
45. Reiss: Ergebn. d. inn. Med. u. Kinderh., 1913, x, 531.
46. Schorer: Cor.-Bl. f. schweiz Aerzte, 1913, xlvii, 1523.
47. Reiss: Ztschr. f. Electrochemie, 1908, xiv, 613.
48. Tuffier and Maute: Tribune med., Paris, 1905.
49. Chiray and Démanche: Compt. rend. Soc. de biol., 1907, lxiii, 235.
50. Schorer: Ueber refraktometrische Pepsin bestimmungen, Diss., Bern, 1908, p. 22.

agreement of results, there being a difference of 1.8 per cent. in one of their results.

Using Reiss' table for the determination of total serum protein, Schorer shows plainly the source of error involved in the former's method.

The protein content of an assumed serum may be supposed to be 8 per cent. If Hammarsten's relation between albumin and globulin is used, then for one part of globulin there is one and a half parts of albumin and 8 per cent. protein would contain 3.2 per cent. globulin and 4.8 per cent albumin.

Refractive index of 1% albumin = .00184 or 4.8% = ...	0.00883
Refractive index of 1% globulin = .00230 or 3.2% = ...	0.00736
Refractive index of total protein = ...	0.01619
Refractive index of distilled water = ...	1.33320
Refractive index of nonprotein = ...	0.00277
Hence the refractive index of whole serum = ...	1.35216

But the refractive index of a serum containing 8% protein according to Reiss' table = 1.3490. Error = 0.0026 or 1.4% protein.

If my average values given in this article for albumin, globulin and nonprotein are substituted for those of Reiss in the above calculations, one obtains an error of about 0.5 per cent. protein, if Reiss' table is used to determine the total protein directly from the refractive index. This error would increase as the globulin and nonproteins increased in disease.

Besides this error there is one due to the too large value for nonproteins adopted by Reiss. This has already been explained in a former paper.† The slight increase in the nonproteins found in the normals in this communication over the value in the former series of normals decreases the percentage of error to approximately 0.4 per cent. instead of 0.5 per cent., which results from the use of the constant value of 0.00277 for nonproteins as advised by Reiss.

It is evident, therefore, that there are two sources of error in the method of Reiss for the estimation of total protein in serum, both of which are removed by Robertson's method. In pathologic cases in which it is known that the nonproteins are not decidedly increased the average value for them given in this paper can be used. If this is done an error of 0.2 per cent. in the albumin content may occur. For clinical work such an error could be allowed, since the technic is greatly shortened if the nonproteins are not estimated in a given serum.

Reiss[51] in his last article acknowledges the correctness of Schorer's criticism, but says that his method is of use in forming a serum pro-

† Footnote 63.

51. Reiss: Deutsch. Arch. f. klin. Med., 1915, cvii, 175.

tein curve for a given patient, by estimating the protein each day, which yields valuable information, especially when combined with the curve of the body weight. He admits that his method is useless in uremia, because of the increase in nonproteins and globulins which occurs in this condition. With Robertson's method, on the contrary, it is entirely possible to make correct determinations of the total protein, albumin, globulin, and nonproteins of uremic serum. This has been done and the results will be published in a separate paper, in which the albumin and globulin content of serums in chronic diseases will be considered.

SYPHILIS

The serum proteins in syphilis have met with considerable attention. Jolles and Oppenheim[52] found no difference in the protein content of normal and syphilitic serum. Klausner[53] was able to demonstrate an increase in the globulin in syphilitic serum by obtaining an abnormal precipitate when distilled water was added to such a serum. Sachs and Altmann[54] thought the Wassermann reaction was due to increased globulin, obtaining their precipitate with weak alcohol. Elias, Neubauer, Porges and Salomon[55] thought the active substance causing the Wassermann reaction belonged to the globulins and that they were present in greater amount in syphilitic than in normal serums. Noguchi[56] found globulin was increased in syphilis and thought it ran so nearly parallel to the Wassermann test that he, for a time, suggested it as a test for syphilis. His estimations were made by direct weighing of the moist globulin precipitate after centrifugalization at five thousand revolutions per minute for a definite time. Gay and Fitzgerald[57] were unable to obtain as definite results as did Noguchi and had better success with the quantitative estimation of euglobulin as a test for syphilis, than with the entire globulin fraction. Muller and Hough[58] found that syphilitic serums showed an increase in the euglobulin, but could establish no relation between the increase in globulin and the Wassermann reaction. Winternitz,[9] by using the method of Hofmeister, Pohl, and Reye, found an increase in the euglobulins in a short series of syphilitics as well as an increase in the total globulins. Moreover, by using the refractometer he demonstrated an increase in the fibrinogen in syphilis. To estimate fibrinogen, he took the difference between the reading of the plasma obtained

52. Jolles and Oppenheim: Ztschr. f. Heilk., 1903, xxiv, 105.
53. Klausner: Wien. klin. Wchnschr., 1908, xxi, 214, 363.
54. Sachs and Altmann: Berl. klin. Wchnschr., 1908, xiv, 522.
55. Elias, Neubauer, Porges and Salomon: Wien, klin. Wchnschr., 1908, xxi, 748, 831 .
56. Noguchi: Jour. Exper. Med., 1909, xi, 84.
57. Gay and Fitzgerald: Boston Med. and Surg. Jour., 1909, clx, 157.
58. Muller and Hough: Wien. klin. Wchnschr., 1911, xxiv, 167.

by the use of hirudin and the reading of the serum. This difference he showed by quantitative chemistry was almost entirely due to fibrinogen.

In the series of nineteen cases in Table 4 the average value for globulin is definitely increased over the normal value, while that for nonprotein is very slightly increased. In Case 1, in which the clinical diagnosis of the early lesion was chancre, the percentage of globulin was found to be normal and the nonspecific nature of the lesion was confirmed by three subsequently negative Wassermann reactions. Cases 3, 6, and 8 were particularly severe and their globulin values

TABLE 5.—ALBUMIN AND GLOBULIN CONTENT OF HUMAN BLOOD SERUM IN PNEUMONIA

Case No.	Date	Diagnosis and Day of Disease	Wassermann	Serum Examination				
				Albumin	Globulin	Total Protein	Nonprotein	Globulin %
1	1/19	Pneumonia, third day.....	Neg.*	3.6	2.7	6.3	1.4	43
2	1/10	Pneumonia, chronic endocarditis	Neg.	...	...	...	...	..
2	1/17	.....	Wk.*	3.8	1.4	5.2	1.4	27
3	1/18	Pneumonia, eighth day.....	Neg.*	3.7	2.3	6	1.5	38
4	1/24	Bronchopneumonia, ninth	Wk.	3.1	2.5	5.6	1.5	45
	2/ 3	.....	Neg.	...	...	...	...	..
5	1/30	Pneumonia, fourth day.....	Mod.	...	...	...	...	..
	1/24	.....	Wk.*	2.9	2.4	6.3	1.6	38
6	1/28	Pneumonia unresolved with arthritis, thirtieth day	Neg.	3.6	3.5	7.1	1.2	49
7	1/28	Pneumonia, third day.....	Neg.	4.7	2	6.7	1.3	30
8	1/31	Pneumonia, delayed resolution tenth day	Neg.*	3.1	3.1	6.2	1.3	50
Averages.....				3.7	2.5	6.2	1.4	40

* Wassermann reactions were done on a sample of the same serum used for estimation of serum proteins.

were found to be among the highest in the series. Unlike other infections considered in this paper, there was no definite decrease in the total proteins.

PNEUMONIA

The literature reviewed in the first part of this paper shows a general agreement over the fact that globulin is increased in pneumonia. Sandelowsky,[59] moreover, has pointed out that the total serum protein falls during the fever and rises gradually during convalescence. An

59. Sandelowsky: Deutsch. Arch. f. klin. Med., 1909, xcvi, 445; ibid., 1910, c, 324.

investigation of the cause of this fall convinced the latter that two types of cases existed:

First, we have those in which the weight remained stationary or increased during fever, decreasing after the fever, while the serum proteins decreased during the fever due to an increase in the water content of the body. Along with this increase in fluid there is a retention of salts, which keeps the osmotic pressure constant and helps to explain the diminished salt in the urine. It was found that only small amounts of salt were contained in the stool and sputum of pneumonic patients and that the supposition that pneumonic exudate contained more salt than normal was not true.

TABLE 6.—Albumin and Globulin Content of Human Blood Serum in Infections Other than Syphilis and Pneumonia

Case No.	Diagnosis	Wassermann	Serum Examination				
			Albumin	Globulin	Total Protein	Non-protein	Globulin %
1	Lung abscess	Neg.*	4.2	3.9	8.1	1.2	48
2	Septic knee	Neg.	3.8	3.4	7.2	1.5	47
3	Pyelitis	Neg.	4.7	2.2	6.9	1.4	32
4	Infected compound fracture	Neg.	4.5	2.1	6.6	1.3	33
5	Recent empyema; acute nephritis	Neg.	3.6	3.4	7	1.6	49
6	Acute mastoiditis; acute nephritis	Neg.	3.1	2.1	5.2	1.5	40
7	Erythema nodosum	Weak+	4	4	8	1.45	50
8	Acute and chronic endocarditis; secondary anemia	Neg.	3.7	2.7	6.4	1.2	42
9	Malignant endocarditis	Neg.	3.8	3.4	7.2	1.5	47
10	Chronic endocarditis; acute arthritis	Neg.	5.7	3.1	8.8	1.3	35
11	Chronic sepsis	Neg.	4.4	2.7	7.1	1.3	38
12	Acute tonsillitis	Neg.	5.8	2.2	8	1.2	27.5
13	Chronic bronchitis	Neg.	5.7	2	7.7	1.3	26
14	Tuberculosis of pleura	Neg.	4.8	2.9	7.7	1.3	38
15	Typhoid, tenth day; Widal +	Neg.	5.4	1.9	7.3	1.3	26

* Wassermann reaction was done on a sample of the serum used for estimation of serum proteins.

Second, we have those in which weight dropped during fever and rose afterwards, while the serum proteins were reduced during the febrile period. Here the consumption of tissues must be accepted as the main cause for the reduced proteins.

The cause for this water and salt retention was thought to be kidney damage, caused mainly by toxins and not caused by fever in itself. Sandelowsky in a later article showed that by producing fever artifi-

cially in dogs the serum proteins were reduced, due to an increase in water in the blood, which he considered possibly a biologic reaction provided against overheating of the body.

Achard, Touraine and Saint-Girons[60] found that in pneumonia, as well as in typhoid, paratyphoid, acute rheumatic fever, phlegmonous sore throat, and streptococcic septicemia, the serum concentration falls until the fever ceases, in some cases, especially in typhoid, to a point higher than normal, after which it drops to the normal level. The most pronounced decrease occurred with typhoid and pneumonia, while only a small decrease came in slight infections.

The results of the examinations in pneumonic serum, as given in Table 5, show a more definite increase in the percentage of globulin than syphilis. The total proteins are decreased moderately, due probably to edema, while the nonproteins are slightly increased. Comment on the accompanying Wassermann reactions will be made in the last section of this paper.

INFECTIONS OTHER THAN SYPHILIS AND PNEUMONIA

The summary of literature under pneumonia applies in large part to the infections to be considered in this section. Though no serum from scarlet fever was examined, it is interesting to include a note about the work of Oppenheimer and Reiss,[61] who showed that the serum proteins decreased, while the body weight increased, during the febrile period. A salt retention was found in this disease, as well as in pneumonia. An attempt was made to determine the presence of nephritis, before the usual signs and symptoms appeared, by following the serum proteins, but without any definite success. Kalser and Löwy[62] found that serum proteins increased after the febrile period, but since they followed no case during the entire febrile period, their conclusion that the serum in scarlet fever is not diluted during the febrile period is not justified.

Most of the cases in Table 6 were of rather medium severity or of considerable duration. It is seen that the total proteins are higher than in cases of pneumonia, though the percentage of globulin is as high. The nonproteins are slightly increased.

Case 5 was one of empyema with little fever and an acute nephritis, with edema confined to the groins. The blood serum is not diluted, though the globulins are markedly increased. If these results are compared to those in Case 6, which was similar except that general edema was present, due to greater kidney damage, as shown by the high

60. Achard, Touraine, and Saint-Girons: Arch. de med. expér. et d'anat. path., 1912, xxiv, 647.
61. Oppenheimer and Reiss: Deutsch. Arch. f. klin. Med., 1909, xcvi, 464.
62. Kalser and Löwy: Deutsch. Arch. f. klin. Med., 1914, cxiv, 82.

TABLE 7.—Effect of the Increase of Globulin by Venous Stasis on the Wassermann Reaction

Case No.	Diagnosis	Normal Serum						Serum after Venous Stasis					
		Albumin	Globulin	Total Protein	Nonprotein	Percentage Globulin	Wassermann	Albumin	Globulin	Total Protein	Nonprotein	Percentage Globulin	Wassermann
1	Obstructive jaundice.....	3.8	2.5	6.3	1.25	40	Mod.	4.7	3.4	8.1	1.3	42	Mod.*
2	Enlarged inguinal glands ?	4.7	2.4	7.1	1.35	34	Neg.*	7	3.4	10.4	1.45	33	Neg.
3	Septic knee.....	3.8	3.4	7.2	1.5	47	Neg.*	5.2	4.2	9.4	1.5	45	Neg.*
4	Aortitis (syphilitic ?); arteriosclerosis; bronchopneumonia; secondary anemia	4.4	3.7	8.1	1.3	46	Neg.*†	5	4	9	1.3	44	Neg.*
5	Normal.....	5.4	1.7	7.1	1.2	24	Neg.*	5.8	2.1	7.9	1.2	27	Neg.*
6	Chronic nephritis.....	3.9	2.4	6.3	1.8	38	Neg.*	5.3	3.4	8.7	1.8	39	Neg.*

*Wassermann reactions were done on a sample of the same serum used for estimation of serum proteins.

† Five days before this examination Wassermann was weak positive. Patient was given mercury and iodid after that time.

value for nonproteins as well as a high nonprotein nitrogen, the effect of increased fluids in the blood is well demonstrated.

Case 12 was one of ordinary tonsillitis of about three days' duration. The values are normal. Case 14 shows a moderate increase in the percentage of globulin, which agrees with the findings of Erben referred to in the first part of this paper. Because of the season of year, only one typhoid serum was examined. The low globulin content is interesting and, if confirmed, will suggest the possibility that increased leukocytes have something to do with increased globulin, as suggested by A. Schmidt, to which suggestion Hammarsten called attention, or that the globulin has a greater resistance to destruction by toxins than has albumin, as suggested by Gottwald.[61]

RELATION OF SERUM GLOBULINS TO THE WASSERMANN REACTION

Literature, as reviewed in the section on syphilis, shows the association that serum globulin has been assumed to have with the Wassermann reaction. But attempts to use the globulin content as a test for syphilis have proved fruitless.

The data which are given in Tables 7 and 8 emphasize the fact that the Wassermann reaction does not depend on a quantitative increase in the serum globulins.

In Tables 4, 5 and 6 the results of the Wassermann reaction, as done in Dr. Wright's laboratory at the Massachusetts General Hospital, are given. The main point of interest is that serums showing a strongly positive test contained on the average considerably less globulin in relation to the total protein than did some showing a negative reaction.

In Tables 5 and 6 several weakly positive Wassermann reactions occurred without any history or any manifestations of syphilis. They were probably examples of spurious reactions, which are known to occur in severe infections. In Case 4 of Table 5 the weak positive became negative during convalescence.

In a recent article the writer[63] has shown that with venous stasis the serum globulin and albumin are proportionately increased. In Table 7 venous stasis has been used to determine whether an actual increase in the globulin content of a serum would increase or cause a positive Wassermann reaction. Wassermann reactions were done on samples of the same serums used in determining the serum proteins. The results show that an absolute increase of 1 per cent. of globulin does not change the results of the fixation test.

Minot and Sellards[64] have shown that negative serums exert an antagonistic action on the Wassermann reaction. The amount of this

63. Rowe, A. H.: Jour. Lab. and Clin. Med., 1916, i, 485.
64. Minot and Sellards: Jour. Med. Research, 1916, xxxiv, 131.

antagonistic reaction can be determined by combining a definite amount of the negative serum with the minimal amount of a positive serum which causes complete fixation, after which a Wassermann test is done. When no antagonistic action is present in the negative serum, no hemolysis occurs, and when such an action is present, hemolysis takes place, up to an absolutely complete degree when the inhibitory power of the negative serum is great. The possibility of studying

TABLE 8.—Relation of Globulin to the Inhibitory Power of Serums Toward the Wassermann Reaction *

Case No.	Diagnosis	Globulin	Percentage Globulin	Hemolysis
1	Syphilis	1.4	18	0
2	Syphilis	2.3	34	0
3	Late syphilis	4.8	45	0
4	Syphilis	2.1	27	0
5	Chronic bronchitis (history of 5 miscarriages)	1.9	29	0
6	Endocarditis; arthritis	3.1	35	0
7	Cholecystitis; syphilis (?)	2.3	32	T
8	Colloid goiter	1.8	28	T
9	Goiter hyperthyroidism	1.1	16	T
Averages		2.3	29.4	...
10	Chronic cholecystitis	2.5	35	P
11	Normal	1.9	29	P
12	Diabetes	2.3	29	P
13	Phthisis; pleurisy	2.9	38	AC
14	Cardiac	1.9	30	AC
15	Goiter; hyperthyroidism	2	27	C+++
Averages		2.25	31.3	...

* All serums in this table were negative to the routine Wassermann reaction. In this table 0 signifies no hemolysis; T, trace of hemolysis; P, partial hemolysis; AC, almost complete hemolysis; C, complete hemolysis.

doubtful negative reactions with this method is evident. Friedemann[65] was able to obtain a positive Wassermann reaction with normal serum globulin, while normal serum albumin prevented fixation. He thought that in normal serum the albumin protects against fixation, while in syphilitic serum the albumin has no such power. Zinsser,[66] in discussing the Wassermann reaction, reviews the work of Schmidt, who

65. Friedemann: Ztschr. f. Hyg. u. Infectionskrankh., 1910, lxvii, 279.
66. Zinsser: Harvey Lectures, 1914-1915, 178: Infection and Resistance, 1915.

states that globulin in syphilitic serum is increased quantitatively and is changed qualitatively and that it probably unites directly with the extract colloids in the antigen of the Wassermann test.

The writer, at the suggestion of Drs. Minot and Sellards, has estimated the globulin in serums on which they had tested the inhibitory power toward the Wassermann reaction. The minimal amount of a positive serum (0.005 c.c. in these cases) which caused complete fixation was added to 0.1 c.c. of each serum tested and a Wassermann reaction was done on each specimen with the results shown in Table 8. It was hoped that a diminution in globulin would occur in serums which inhibited the Wassermann reaction, whereas a high globulin content would be found in those in which no inhibitory power was present.

The results in Table 8 show an average value of 29.4 per cent. for the globulin in serums causing a trace or no hemolysis, while the average of the values in serums with partial, almost complete, and complete hemolysis is slightly higher, being 31.3 per cent. More estimations on serums causing complete hemolysis must be made before any definite conclusions can be drawn as to whether all serums showing complete hemolysis will have low globulin content.

The data given in the last section of this article lead to the conclusion that the Wassermann reaction is not due to a quantitative increase in the serum globulins.

SUMMARY

A series of normal cases shows that serum albumin varies between 4.6 and 6.7 per cent., that globulin varies between 1.2 and 2.3 per cent., that total proteins vary between 6.5 and 8.2 per cent., that nonproteins vary between 1.1 and 1.3 per cent., while the percentage of globulin in the total protein varies from 16 to 32 per cent. The average value for albumin was found to be 5.6 per cent. for globulin, 1.9 per cent., for total protein 7.5 per cent., for nonprotein 1.24 per cent. and for globulin 25.5 per cent.

The normal values for total protein, albumin and globulin from the entire literature are given, while the literature on these proteins in health and disease as well as on methods of their estimations is summarized.

The former use of the refractometer in medical research is discussed, while the error in Reiss' method for the determination of total proteins is pointed out. It is shown that Robertson's microrefractometric method for the determination of total protein is free from evident error and for the estimation of albumin and globulin is the most satisfactory method yet proposed.

In syphilis the globulin is definitely increased, while the total protein remains about normal.

In pneumonia the globulin is increased more in relation to the total protein than in syphilis, while the total protein is reduced, due, probably, in large measure to a dilution of blood serum by water retention, which occurs in fever.

In many chronic septic conditions, in mild infections and typhoid, the total protein is not decreased, as it is in pneumonia. Globulin seems definitely increased in all infections, except in acute tonsillitis, typhoid, and in certain mild infections, such as chronic bronchitis. The marked dilution of serum which occurs with anasarca is shown in two cases of acute infection associated with acute nephritis, which were investigated.

The evidence presented shows that the Wassermann reaction is not due to a quantitative increase in the serum globulins.

I wish to express my sincere gratitude to Drs. Edsall and Dennis for advice given during this research. I also appreciate the opportunity of using the ward cases on the services of Drs. Lee, Lord, Pratt, and William Smith, and the cases in the outpatient departments of Drs. Richard Cabot and C. Morton Smith.

THE BLOOD PLATELETS IN HEMOPHILIA*

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Hemophilia on account of its striking nature was early recognized as a clinical entity, and has been the subject of many observations. Many theories as to its causation have been advanced. With the development of methods for the study of the blood it has been possible to discard most of the theories concerning this interesting disease.

It has been conclusively shown that in typical cases the numerical counts of the formed elements of the blood, the erythrocytes and the leukocytes are within normal limits. The blood platelets may be somewhat increased. It has also been shown in typical cases that certain active principles of the blood that participate in clotting occur in normal amounts. The hemophilic blood serum[1] contains the normal amount of thrombin, the active coagulating principle, and this thrombin behaves in an entirely normal fashion. It has further been shown that the hemophilic blood clot, when once formed, is as tough and firm as normal and retracts in normal fashion. The fibrinogen[2] and calcium[3] are considered to be within normal limits.

It has been suggested that the presence of an excess of some anticoagulating substance was the cause for the long coagulation time exhibited by hemophilic blood. Weil[4] has given some experimental evidence that an excess of antithrombin occurs in hemophilic blood. Howell,[5] however, was not able to find that his antithrombin was notably increased in hemophilia. The findings of Hurwitz and Lucas,[6] Hess[7] and our studies confirm Howell's findings in regard to antithrombin.

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1. Fonio: Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1914, xxviii, 313. Addis: Jour. Path. and Bacteriol., 1911, xv, 427. Gressot: Zeschr. f. klin. Med., 1912, lxxvi, 194. Morowitz and Lossen: Deutsch. Arch. f. klin. Med., 1908, xciv, 110.
2. Addis: Jour. Path. and Bacteriol., 1911, xv, 427. Sahli: Ztschr. f. klin. Med., 1905, lvi, 264; Deutsch. Arch. f. klin. Med., 1910, xcix, 518. Hurwitz and Lucas: THE ARCHIVES INT. MED., 1916, xvii, 543.
3. Addis: Jour. Path. and Bacteriol., 1911, xv, 427. Nolf: Ergebn. d. inn. Med. u. Kinderh., 1913, x, 275. Morowitz and Lossen: Deutsch. Arch. f. klin. Med., 1908, xciv, 110. Hess: Bull. Johns Hopkins Hosp., 1915, xxvi, 372.
4. Weil: Bull. et mém. Soc. méd. hôp. de Paris, 1906; Presse méd., 1905, xiii, 673.
5. Howell: THE ARCHIVES INT. MED., 1914, xiii, 76.
6. Hurwitz and Lucas: Footnote, 2, last reference.
7. Hess: THE ARCHIVES INT. MED., 1916, xvii, 203.

Morawitz and Lossen[8] and Sahli[9] are inclined to attribute the faulty coagulation to an insufficiency or defective formation of the element known in various terminologies as thrombokinase, thromboplastic substance,[10] tissue juice, cytozyme or thrombozyme. This is similar to the view of Nolf and Herry,[12] who believe that there is a quantitative or qualitative deficiency in the thrombozyme, which occurs in the blood platelets in the circulating blood in contrast to the similar factor in the tissues. These various substances mentioned above are not exactly identical in the various terminologies, but can be considered roughly comparable and are found in the blood platelets and tissue juices. The action ascribed to them varies according to the theories of coagulation; all theories hold in common that they accelerate the coagulation of the blood.

Addis[13] believes that the abnormality of hemophilic blood is to be found in the property of the prothrombin, that it is altered in character so that it requires a longer time than normal for its activation to thrombin. Howell[5] concludes from his experiments that it is the actual amount of prothrombin that is altered in the hemophilic blood and is inclined to believe that it is the deficiency in the amount of prothrombin with the resulting relative excess of antithrombin that is responsible for the abnormally long coagulation time. He does not deny to the blood platelets a share in this deficiency.

The action of blood platelets from cases of hemophilia has received but little study and forms the basis of this paper.

Fonio,[14] who believes that the active coagulating principle in the tissues, while similar, is not identical with that of the blood platelets, is inclined to attribute the delayed coagulation in hemophilia, from the study of one case, to an insufficiency of thrombozyme or blood platelets, which are present in sufficient amounts. Sahli[15] in 1910 demonstrated similar differences with hemophilic and normal red blood cells as Fonio did with platelets. His results, perhaps, were due to platelets contained in his red cell suspensions. Addis[13] has discussed Sahli's results.

The experiments given below were made with the blood platelets obtained from two typical cases of hemophilia and show clearly that hemophilic platelets do not act like normal platelets. The platelets of a third case of hemophilia, a mild case, gave the same, but less striking, results.

8. Morawitz and Lossen: Footnote 1, last reference.
9. Sahli: Footnote 2, second reference.
10. Howell: Am. Jour. Physiol., 1912-1913, xxxi, 1.
12. Nolf and Herry: Rev. de méd., 1909, xxix, 841; 1910, xxx, 20 and 106.
13. Addis: Footnote 1, second reference.
14. Fonio: Cor.-Bl. f. schweiz. Aerzte, 1915, xlv, 1505; Footnote 1, first reference.
15. Sahli: Footnote 2, second reference.

METHODS

Plasma was obtained from the hemophilic blood without adding any oxalate or other foreign substance, by drawing the blood from the arm vein into a paraffined syringe and centrifugalizing it, by preference, in iced paraffined tubes. A clear plasma containing very few platelets could easily be obtained, which did not clot for twelve hours. The cloudier the plasma, that is, the more platelets it contained, the quicker it clotted. Platelet suspensions in normal salt solution were obtained from the "buffy coat" and from the cloudy plasma of the hemophilic blood by the usual technic described first by Mosen[16] and later modified by us.[17] Platelets were obtained also from the cloudy plasma and "buffy coat" of oxalated, citrated, and magnesium sulphate normal and hemophilic plasmas. The results with the hemophilic platelets were essentially the same irrespective of the particular method of obtaining them or the number of washings. The same was true of the normal platelets.

ACTION OF PLATELETS ON HEMOPHILIC PLASMA

The effect of adding suspensions of hemophilic and normal platelets to hemophilic plasma is shown in Tables 1, 2 and 3.

Hemophilic plasma clots most rapidly on the addition of normal platelets. Roughly, the time varies inversely to the number of platelets. Yet an increase of platelets above a certain amount, relatively small, does not greatly shorten the time. Hemophilic platelets hasten somewhat the clotting of hemophilic plasma, although nowhere nearly as markedly as normal platelets in the same concentration. In each instance the clotting time of the mixture of hemophilic plasma and hemophilic platelets in any concentration of platelets is always markedly longer than the clotting time of the mixture of hemophilic plasma and normal platelets. Even when the mixture contains seventy-five times as many hemophilic platelets as ordinarily occur in the circulating blood, the clotting time of the mixture is abnormal and markedly longer than the clotting time of a mixture of hemophilic plasma and normal platelets in which the platelets are approximately in the same number as in the circulating blood. The same results were obtained whether the platelets from one hemophilic case were added to its own or to another hemophilic plasma. The platelets obtained from the hemophilic case with the longest coagulation time usually, but not always, required a somewhat longer time to cause a hemophilic plasma to clot than those from the case with the shorter coagulation time. An example of this is given in Table 3. These studies were repeated many times.

16. Mosen: Arch. f. Anat. u. Physiol., 1893, Physiol. Abst., p. 352.
17. Lee and Vincent: THE ARCHIVES INT. MED., 1914, xiii, 398.

TABLE 1.—Effect of Adding Hemophilic and Normal Platelets to Hemophilic Plasma

Strength of Platelet Suspension	Variety and Amount of Platelet Suspension Added to 6 Gtt. of Hemophilic Plasma	Time Coagulation Begins, Minutes	Time for Solid Clot to Form, Minutes
Saturated	Normal 2 gtt.	4	6
	Normal 3 gtt.	4	5
	Hemophilic 2 gtt.	21	42
	Hemophilic 6 gtt.	13	23
Platelets about four times as concentrated as in blood	Normal 2 gtt.	5	8
	Normal 3 gtt.	5	7
	Hemophilic 2 gtt.	38	55
	Hemophilic 6 gtt.	38	55

TABLE 2.—Effect of Adding Hemophilic and Normal Platelets to Hemophilic Plasma

Strength of Platelet Suspension	Variety and Amount of Platelet Suspension Added to 6 Gtt. of Hemophilic Plasma	Time Coagulation Begins, Minutes	Time for Solid Clot to Form, Minutes
About twice as concentrated as in blood	Normal 3 gtt.	19	24
	Hemophilic 3 gtt.	77	120
About eight times as concentrated as in blood	Normal 2 gtt.	18	22
	Hemophilic 2 gtt.	55	95
	Hemophilic 11 gtt.	52	85
About sixteen times as concentrated as in blood	Normal 2 gtt.	16	21
	Hemophilic 2 gtt.	47	72
Saturated solid mass of pure white platelets	Normal 2 gtt.	15	20
	Hemophilic 2 gtt.	50	76
	Hemophilic 10 gtt.	35	50

TABLE 3.—Effect of Adding to the Same or Different Hemophilic Plasmas Platelets from Two Different Cases of Hemophilia *

Strength of Platelet Suspension	Variety and Amount of Platelet Suspension Added to 6 Gtt. of Hemophilic Plasma	Time Coagulation Begins, Minutes	Time for Solid Clot to Form, Minutes
Saturated	Hemophilic 1, 1 gtt.	54	70
	Hemophilic 2, 1 gtt.	41	70
	Hemophilic 1, 12 gtt.	48	65
	Hemophilic 2, 12 gtt.	20	35

* No. 1 had a shorter coagulation time than No. 2.

Usually, as shown in Table 1, the normal platelets allowed the hemophilic plasma to clot as rapidly as normal blood; occasionally the normal platelets did not shorten the time of a particular plasma quite so much (Table 2). This may have been due to a poor suspension of normal platelets, since it is possible that in collecting them some of their activity was destroyed. However, a heavy solution of cephalin (Howell's thromboplastic substance[10]) added to the hemophilic plasma acted similarly to the normal platelet suspension; that is, if the normal platelets in concentration could shorten the coagulation time of the hemophilic plasma only to twelve minutes, then cephalin could shorten it only to ten or twelve minutes; while if the normal platelet suspension shortened the coagulation time to four minutes, cephalic shortened it to three or four minutes. Such differences seem to have been due to differences in the plasma on the different days. It is possible that the difference was due to some slight alterations in the technic in collecting the blood which were not appreciated. A reasonable interpretation of this difference is the presence of some antagonistic substance in the plasma itself. This substance may be constantly present, but may have been detected only under favorable circumstances. It seems somewhat distinct from the platelets, but may be derived from them.

ACTION OF PLATELETS ON RECALCIFIED OXALATED PLASMA

The Effect of Adding Platelets to Hemophilic Recalcified Oxalated Plasma.—Table 4 shows the results obtained on adding normal and hemophilic platelets to hemophilic oxalated plasma and then adding varying amounts of calcium. This table shows that the normal platelets, even in small amounts, are able to accelerate the clotting time of recalcified oxalated hemophilic plasma more than hemophilic platelets, even when the latter are added in great concentration. This difference between the action of the hemophilic and normal platelets is quite similar to their different action seen when they were added to the unoxalated hemophilic plasma. It is to be noted that if one uses an optimum amount of calcium to recalcify the hemophilic oxalated plasma the difference between adding normal and hemophilic platelets is not so great as when the amount of calcium used is below the optimum. Also it is to be noted that the time of beginning coagulation in the presence of the hemophilic platelets may be nearly the same as in the presence of normal platelets when the optimum amount of calcium is used. However, the time from the appearance of the first fibrin strands to the point at which the clot is solid, is shorter when normal rather than when hemophilic platelets are added. The most striking results are seen when but little calcium is added. In such instances, when one adds one quarter as many normal as hemophilic platelets, a solid clot is formed some minutes before any coagulation is evident in the tube

containing four times as many hemophilic platelets, which never becomes solid. We interpret these results as follows: Under the most favorable conditions the hemophilic platelets act fairly well, but not so well as normal platelets. Under unfavorable conditions the hemophilic platelets act poorly and some of them probably not at all, so that not enough thrombin is formed or rendered available to complete coagulation.

TABLE 4.—Effect of Normal and Hemophilic Platelets on the Clotting Time of Recalcified Hemophilic Oxalated Plasma

Strength of Platelet Suspension Added	Variety and Amount of Platelet Suspension Added to 6 Gtt. of Hemophilic Oxalated Plasma	4 Gtt., 5% $CaCl_2$		3 Gtt., 5% $CaCl_2$		2 Gtt., 5% $CaCl_2$	
		Coagulation Begins, Min.	Time for Solid Clot to Form, Min.	Coagulation Begins, Min.	Time for Solid Clot to Form, Min.	Coagulation Begins, Min.	Time for Solid Clot to Form, Min.
About four times as concentrated as in blood	Normal 2 gtt.	7	10	18	25	Sliding jelly clot, 30	Not in three hours
	Normal 5 gtt.	6	9	10	16	Sliding jelly clot, 25	Not in three hours
	Hemophilic 2 gtt.	8	16	30	Never	Never	Not in 3 hours
	Hemophilic 5 gtt.	8	15	17	36(never firm)	Never	Not in 3 hours
Saturated.....	Normal 2 gtt.	4	7	5	7	7	11
	Hemophilic 2 gtt.	8	15	14	Sliding jelly clot 30, never solid	Never	Not in three hours
	Hemophilic 4 gtt.	8	15	14	Sliding jelly clot 30, never solid	Tiny strands 25	Not in three hours
	Hemophilia 8 gtt.	7	14	18	24, never as solid as with normal platelets	Weak sliding jelly, 19	Not in three hours
0	0	?	60	?	Never	?	Not in 3 hours

The Effect of Adding Platelets to Normal Oxalated Plasma.—Table 5 is similar to Table 4 and shows the effect of adding normal and hemophilic platelets to oxalated normal plasma, which is then recalcified. The difference between the action of the hemophilic and normal platelets on recalcified normal plasma is much more difficult to demonstrate than on hemophilic plasma, even using an amount of calcium below the optimum. Better results were obtained with a plasma that had stood forty-eight hours than with plasma a few hours old. With an optimum amount of calcium no especial difference could be

seen between the accelerating action of normal and hemophilic platelets. With an amount of calcium just below the optimum often there occurred no essential difference in the time that the clots formed in the presence of the two kinds of platelets. In general, the clotting time seemed a shade longer with hemophilic platelets. However, it was always noticed that the clots formed in the presence of the hemophilic platelets were at the time they were solid, distinctly of a weaker nature than those containing normal platelets. Later they became as firm as those with the normal platelets.

By using a suitable amount of calcium that could only be determined for each oxalated plasma by many trials we got the results given in Table 5, under two drops of calcium, where the difference between adding hemophilic and normal platelets is clearly demonstrated. Here, though coagulation at times began at about the same

TABLE 5.—EFFECT OF NORMAL AND HEMOPHILIC PLATELETS ON THE CLOTTING TIME OF RECALCIFIED NORMAL OXALATED PLASMA

Strength of Platelet Suspension	Variety and Amount of Platelet Suspension Added to 6 Gtt. of Normal Oxalated Plasma	Time in Minutes for Solid Clot to Form, with 5% Solution $CaCl_2$ Added		
		4 Gtt.	3 Gtt.	2 Gtt.
About four times as concentrated as blood	Normal 1 gtt.	4	7	9
	Normal 5 gtt.	3+	4—	5+
	Hemophilic 1 gtt.	4+	10*	Never
	Hemophilic 5 gtt.	4	4+*	Begins 10, never solid
Saturated.....	Normal 1 gtt.	4—	4—	5
	Hemophilic 2 gtt.	4+*	4+*	6+, never solid
0	0	8	Begins 9, never solid	Few strands 25, never solid

* Though solid at the time given, these clots were not so firm as those containing normal platelets and did not become any firmer.

time, no matter whether hemophilic or normal platelets were added, the latter allowed a solid clot to form, while the plasma with the hemophilic platelets never became solid. Thus the beginning coagulation time may be near the same time with either kind of platelets, while with hemophilic platelets the ending coagulation time is much delayed.

We assume that only under unfavorable conditions can marked differences be detected between the action of hemophilic and normal platelets on normal plasma. Ordinarily in oxalated normal plasma recalcification makes favorable conditions. As we shall bring out later, our microscopic studies show that hemophilic platelets under favorable conditions undergo transformation not very differently from normal platelets. Irrespective of any possible antagonistic substance in the hemophilic plasma, it is very different from normal oxalated plasma. Hemophilic plasma may well be compared to an oxalated normal

plasma collected by the most careful technic, as described previously by us.[17] Such a plasma, in which the greatest care is exercised to prevent any of the changes incident to coagulation, will clot on recalcification with the optimum amount of calcium in forty minutes or more. Under the usual conditions of technic of preparing an oxalated plasma, the plasma will clot upon recalcification in fourteen minutes or less. Under the latter conditions the earlier changes incident to the process of coagulation have already begun and the plasma may be said to be partially prepared for clotting before recalcification. Hemophilic plasma can be readily obtained relatively free from these preliminary changes. On the other hand, normal plasma can be so obtained only by the exercise of special precautions. We have already shown elsewhere that these precautions in normal plasma result in the preservation of intact platelets. In hemophilic plasma the platelets, seemingly fixed, require no such elaborate precautions.

ABILITY OF HEMOPHILIC PLATELETS TO FORM THROMBIN ACCORDING TO THE THEORY OF BORDET AND DELANGE

In order further to determine the difference between hemophilic and normal platelets the ability of each to form thrombin was tested. This was done according to Bordet and Delange's[18] method. They believe that thrombin is formed by the reaction between cytozyme (platelets and other tissue juices) and serozyme (similar to prothrombin) in the presence of soluble calcium salt. Serozyme is the defibrinated serum obtained after a very clear oxalated plasma, free as possible from platelets, has been clotted by recalcification and kept for twenty-four hours to get rid of any active thrombin. Such a thrombin requires about six or seven minutes to form. The addition of oxalate to a mixture of platelets and serozyme and calcium not only precipitates the calcium and prevents the further formation of thrombin, but also has a deterrent effect on the thrombin.[17] It is thus possible to demonstrate the amount and activity of a thrombin thus formed within any given time and furthermore to determine the rapidity with which thrombin is formed, since thrombin and fibrinogen react quantitatively to a certain degree, especially if dealing with minute amounts of thrombin.

Table 6 is a typical protocol of often-repeated experiments. For these experiments serozyme, thrombin-free platelets, calcium solution, and a fibrinogen solution were used. The serozyme was demonstrated to be free of active thrombin in that it did not clot the fibrinogen solution in twenty-four hours. The action of normal and hemophilic

18. Bordet and Delange: Ann. de l'Inst Pasteur, 1912, xxvi, 657 and 737. Lee and Vincent: Footnote 17.

serozyme was the same. In the tests, to one drop of serozyme was added 0.5 c.c. of a 1 to 100 aqueous solution of calcium chlorid and then suspensions of platelets. The platelets were used in amounts over ten times the minimum required to form enough thrombin to clot the fibrinogen solution in three minutes. After the calcium, serozyme, and platelets had been in contact either six or eight minutes, varying amounts of a 1 per cent. solution of sodium oxalate were added to inhibit the action of forming more thrombin and to paralyze what thrombin had been formed. Then, either immediately or after an interval not exceeding four minutes a fibrinogen solution was added and the time a clot formed was noted. If there was an excess of thrombin formed, the effect of adding a small amount of oxalate did not alter the coagulation time with the fibrinogen solution. If a small amount of thrombin was formed, it paralyzed this small amount and delayed the reaction between thrombin and fibrinogen.

TABLE 6.—Ability of Normal or Hemophilic Platelets to Form Thrombin According to Bordet and Delange's Method*

Drops of Saturated Suspension of Platelets	Amount of 1% Solution of Sodium Oxalate Added, Gtt.	Clotting Time, Minutes
Normal ¼ or 1	0	3
Hemophilic ¼ or 1	0	3
Normal 1	½	3
Hemophilic 1	½	16
Normal 1	1	5
Hemophilic 1	1	22
Normal 1	2	25
Hemophilic 1	2	About 4½ hours

* In each of the preparations there was 1 gtt. of serozyme and 0.5 c.c. of dilute (1 to 100) calcium chlorid solution, to which was added the amount of platelets indicated; then after an interval of six or eight minutes in each instance there was added the amount of oxalate solution given, then either immediately or after waiting not more than four minutes more 6 gtt. of a fibrinogen solution was added; clots forming in the time given.

By referring to Table 6 it is seen that a sufficient amount of hemophilic platelets can form enough thrombin to clot fibrinogen in the same time as thrombin formed by normal platelets, provided adequate time was given for the formation of thrombin. However, it is seen that by the addition of oxalate after thrombin has been formed, it takes a much longer time for the thrombin formed by the hemophilic platelets than by the normal platelets to clot the fibrinogen, even in some instances eighteen times as long. This is best explained by the fact that there is a slow formation of thrombin by the hemophilic platelets and that in the given time they have formed less thrombin. That

there is plenty of thrombin eventually formed has often been shown, since hemophilic serum contains fully as much thrombin as normal. We assume therefore that thrombin is formed more slowly.

To demonstrate further that these hemophilic platelets were slow thrombin formers we used varying dilutions of hemophilic and normal platelet suspensions. It was found that a thrombin formed with one drop of a very weak suspension of hemophilic platelets required a much longer time to clot fibrinogen than when an equal suspension of normal platelets was used. Similar results were obtained with weak, but more concentrated, suspensions. In such an experiment we deal with the great difficulty of making equal platelet suspensions and being able to say they are truly equal. Since the hemophilic blood contained more platelets and yielded platelets more easily, the error favored the hemophilic rather than the normal suspension. However, whether one

TABLE 7.—Difference Between Adding Hemophilic Platelet Extract and Suspension to Hemophilic Plasma

Amount of Hemophilic Plasma	Character of Suspension or Extract of Hemophilic Plates	Amount of Extract or Suspension Added, Gtt.	Coagulation Begins, Min.	Coagulation Intermediate, Min.	Coagulation Ends, Min.
6 gtt.	0	0	360	?	?
6 gtt.	1 gtt. of saturated suspension and 5 gtt. of salt solution	6	50	80	150
6 gtt.	1 gtt. of extract of same saturated suspension	3	12	20	30
	The same with 9 gtt. of water	6	10	17	26

uses a few platelets to form thrombin, or allows a large number of platelets to form what thrombin they can and then this is paralyzed by oxalate, one arrives, of course, at the same results. The actual experimental results are the same and show that the hemophilic platelets act much more slowly in forming thrombin than normal platelets.

In these experiments the fibrinogen solution used consisted of one part oxalated plasma, eight parts 0.9 per cent. salt solution and 0.5 part of 1 per cent. sodium oxalate in 0.9 per cent. sodium chlorid. Both hemophilic and normal oxalated plasma were used. If hemophilic fibrinogen solution was used to clot the thrombin formed by the interaction of serozyme, calcium and cytozyme, the clots required a slightly longer time to form than if normal fibrinogen was used. This was probably due to the fact that the normal plasma contained a few platelets which acted to accelerate the reaction more than the very few hemophilic platelets in their plasma.

STUDIES ON THE WHOLE BLOOD

Addition of Platelets to Whole Blood.—Blood was drawn from the arm vein of a hemophilic patient and 1 c.c. put in each of a series of tubes. One c.c. of this blood coagulated in fifty-five minutes. The addition of one drop of a weak suspension of fresh normal platelets caused this blood to clot in fifteen minutes, while two drops of a correspondingly weak suspension of hemophilic platelets caused the blood

TABLE 8.—COAGULATION TIMES OF WHOLE BLOOD, THE PROTHROMBIN TIME, AND ANTITHROMBIN FACTOR; THE EFFECT OF TRANSFUSION ON THE COAGULATION TIME

Date	Coagulation Time, Min.	Prothrombin Time, Min.	Prothrombin Control, Min.	Antithrombin Factor	Remarks
Case 1					
March 10	55	..	..	....	
March 13	70	50	10	1+	No bleeding or change in patient's condition
March 19	40	28	8	1.5	
March 23	70	50	8	1.35	
March 26	50	45	10	1.2	
Case 2					
March 19	100	70	8	1.25	
March 21	60	49	12	1.3	No change in patient's condition or bleeding
March 23	92	60	10	1.15	
March 31 (before transfusion)	150	85	12	.....	
March 31 (after transfusion)	8	..	..	.....	March 31 transfused 600 c.c. of blood from donor, whose coagulation time was 7
March 31 (6 hours after transfusion)	10	..	..	.....	
April 1	17	..	..	....	
April 2	25	..	..	.....	
April 3	60	..	..	.....	April 7 spontaneous hemorrhage into right knee joint
April 5	100	..	..	.....	
April 12	55	..	..	.....	
April 14	115	..	..	.....	
April 20	100	..	..	.....	

to coagulate in forty minutes. One drop of a "saturated" suspension of normal platelets caused coagulation in five minutes and double this amount of hemophilic platelets caused coagulation in twenty-five minutes. The first fibrin strands in the tubes containing the hemophilic platelets appeared from one to four minutes after they appeared in those with the normal platelets, but coagulation proceeded to completion much more slowly. It is important in order to get these wide differences to use fresh preparations of platelets. We have found that

the hemophilic platelets on standing for over twenty-four hours seem to undergo some change, since in some instances these old hemophilic platelets acted more rapidly than the freshly prepared platelets. The results with hemophilic and normal bloods were consistent with those obtained with the hemophilic plasma, oxalated hemophilic, and normal blood plasma.

Studies on the Coagulation Time of the Whole Blood.—The coagulation time of the whole blood was determined by the method of Lee and White,[19] which is to allow 1 c.c. of blood drawn from a vein to clot firmly in a tube eight mm. in diameter. By this procedure normal blood clots in five to ten minutes. The results are seen in Table 8, and show the usual prolongation of the coagulation time of hemophilic blood. Fluctuations occurred without evident reason, such as has been noted by others.

Case 1.—A man, 50 years of age, and the man in Case 2, who was 25 years of age, both gave a clean cut family history of hemophilia and had suffered from nose bleeds and severe bleeding from injuries. One gave a history of hematuria. Both had suffered since childhood with repeated attacks of swelling, stiffness and pain in the various joints of the body and the joints gave the typical appearance of chronic hemophilic joints. In Case 1 there had been no acute joint swelling or pain for three months before entrance, but the patient had had some bleeding from his gums six weeks previously, associated with carious teeth. In Case 2 there was an acute swelling of the knee some two months before entrance, but the patient had had no other hemorrhagic symptoms for over a year. During his stay in the hospital he developed again acute swelling and pain in his knee, subsiding after ten days. Otherwise during the present study of these cases there was no evidence of bleeding.

Case 2 is of especial interest. In view of the fact that normal platelets in small amounts in vitro could shorten the hemophilic coagulation time, it was thought that the transfusion of normal blood might shorten the coagulation time of the hemophilic blood, and that the lasting effect of such a procedure would be evident as long as the life of the normal transfused platelets. To test this point, with the enthusiastic approval of the patient Dr. Vincent transfused the second patient with 600 c.c. of blood from a donor of the same iso-agglutination group, whose coagulation time was seven minutes and whose platelets were normal in numbers. Before the transfusion 500 c.c. of blood were removed from the patient. Allowing that the patient's and donor's blood volume was 5 liters, the number of normal platelets that were given this patient would be about 24,000 per c.c.

The coagulation time one and one-half hours before transfusion was 150 minutes. On this same day directly before transfusion the coagulation time determined by obtaining blood through a paraffined cannula and allowing it to run directly into the glass coagulation time tube was four and one-half hours. It was quite striking to find directly after the transfusion that the hemophilic blood clotted in normal time, as it also did six hours after transfusion. There was a definite gradual lengthening of the coagulation time, so that it was sixty minutes three days, and 100 minutes five days, after the transfusion.

Thus the effect of the transfusion was not evident after three days. This time coincides with our ideas of the life of the platelets, as pointed

19. Lee and White: Am. Jour. Med. Sc., 1913, cxlv, 495.

out by Duke.[20] It seems reasonable to suppose that the effect of the transfusion was due to the normal platelets, that as they began to "die" the coagulation time lengthened and kept on doing so until its previous delayed time was reached, at which time all the normal platelets had died off.

Character of the Blood Clot.—The clots of the whole blood always retracted fully as much as normal clots and gave the typical appearance of hemophilic clots, that is, white on top, red below, with greater retraction at the top of the clot because of the richness of the platelets in this region. Clots from clear plasma did not retract unless a sufficient amount of platelets had been added; these clots never seemed to retract as well as blood or cloudy plasma, containing numerous platelets which had not been first removed.

If after the hemophilic blood had apparently clotted firmly the clot was loosened and removed from the fluid, the fluid would clot and would do so sometimes almost at once, often in three to five minutes and sometimes not for ten to thirty minutes or more. Again on separating the clot, but not removing it from the serum, this reclotting phenomenon occurred and might be repeated from three to six times. The reclotting would occur no matter whether the clot was loosened a few minutes or a few hours after it had appeared solid. If one waited hours rather than minutes after the clot had formed, one usually obtained the reclotting phenomenon fewer times. This same phenomenon was observed on clotting hemophilic oxalated plasma with calcium to obtain serozyme. (It was first called to our attention by Dr. Howell.) This phenomenon has been observed in a number of cases of hemophilia and in other cases with delayed coagulation time and prolonged prothrombin time. Of some twenty-five observations on eight cases which have exhibited this phenomenon, none have shown an antithrombin content below one,[21] while almost all the observations have shown an increase in antithrombin. It was thought, as suggested by Dr. Howell, that the increased antithrombin content had something to do with this phenomenon. This phenomenon is also seen in preparing serozyme from very clear normal oxalated plasma. An explanation which now occurs to us is that the phenomenon is associated with a slow formation of thrombin. Thrombin may be slowly formed from at least two causes traceable to blood platelets. In the case of hemophilia the blood platelets, while sufficiently numerous, are not readily available for purposes of coagulation, hence a slow formation of an ample amount of thrombin. In the case of purpura hemorrhagica associated with a low blood platelet count we may have a slow formation due to the paucity of blood platelets. Under normal conditions

20. Duke: Jour. Exper. Med., 1911, xiv, 265.
21. Minot, Denny and Davis: THE ARCHIVES INT. MED., 1916, xvii, 101.

there is probably an excess of blood platelets, and as several observers have elsewhere shown, the excess of blood platelets over the number actually required for coagulation acts in retracting the clot. In purpura hemorrhagica there is no clot retraction, since all the blood platelets are used for thrombin formation, which is relatively slow due to the necessity of using all the blood platelets.

FURTHER STUDIES ON PLATELETS

In these two cases of hemophilia the platelet count determined by Wright and Kinnicutt's[22] method was from 290,000 to 400,000, that is at or slightly above the upper limit of normal.

The Bleeding Time.—The time a patient bleeds from a puncture in the ear has been shown by Duke[23] to be prolonged in conditions with very few platelets. In such conditions, however, the coagulation time is often normal, although it may be somewhat delayed. It seems that in such cases there is enough thrombin to allow the blood to clot in normal time, or not greatly beyond the normal time, though in our experience a weak clot occurs. One must realize that it probably takes very few normal platelets to do their share in allowing blood to coagulate in normal time and that a little thrombin can cause fibrinogen to clot, though weakly, in the same time as much larger amounts; while with minimal amounts of thrombin the time is delayed and the clot is weak. Duke tested the bleeding time of but one case of hemophilia and found it normal. Other observations have recently been made by Hess,[7] who found this time normal in several hemophilic cases, which is in accord with our repeated findings in these two cases and also in three others of typical hemophilia. The bleeding time from an arm vein was also normal in these cases. It is rather surprising that typical cases of hemophilia, in which bleeding occurs readily after cuts, injuries, etc., and which show a marked delay in the coagulation time of the blood, do not always have an abnormal bleeding time. Whether this is to be considered entirely due to the fact that their platelets are plentiful in numbers will be referred to later.

Studies on the Metamorphosis of the Platelets.—Under the microscope we have repeatedly observed the agglutination and fusion into glassy-like masses and often dissolution of the platelets that occurs when any serum containing active thrombin and calcium is allowed to act on platelets. The action of antithrombin, oxalated plasma, pure thrombin, etc., and combinations of such blood elements on blood platelets is reserved for a future communication. We were, however, unable to detect that hemophilic or normal serum, antithrombin, serozyme, plasma, pure thrombin and calcium and various combinations of

22. Wright and Kinnicutt: Jour. Am. Med. Assn., 1911, lvi, 1457.
23. Duke: The Archives Int. Med., 1912, x, 445.

such substances acted differently on hemophilic than on normal platelets. No matter whether the platelets were washed one or more times or whether they were obtained from oxalated plasma or magnesium sulphate plasma. Whatever permitted the metamorphosis of the hemophilic or normal platelets did so as readily and quickly with hemophilic as with normal coagulation elements. Dr. J. Homer Wright could find no particular histologic difference in the fused masses of hemophilic and normal platelets, whether caused by hemophilic or normal serum acting on the platelets for a longer or shorter period.

It is noteworthy that no fusion or microscopic change occurred in the platelets in the hemophilic plasma or blood until fibrin strands began to form from thirty to forty minutes after withdrawing the blood, which clotted firmly in seventy minutes. Practically all the platelets were more or less fused, some more than others, five to eight minutes after the process began.

The platelets from the depth of the red cell sediment obtained after centrifugalizing twenty minutes the whole blood and after the plasma was removed appeared clumped, but not fused. After some hours these platelets appeared the same, though the plasma had clotted. After twenty-four hours they appeared disintegrated, rather than fused, and the red cell sediment showed no clot.

It has often been suggested that the platelets are important factors in the coagulation of the blood and that they have something to do with the initial stage of coagulation. In most theories it is agreed that immediately as blood is shed it undergoes some change which makes it quite different from normal circulating blood and enables coagulation to ensue. The duration of this stage is probably short, but concerning it very little is known. It is perhaps significant that with the first visible signs of coagulation the platelets begin to undergo this metamorphosis. Perhaps before coagulation and metamorphosis of the platelets can properly proceed, certain substances in the platelets must go into solution. Such substances derived from the platelets probably occur in solution in the circulating blood.

ULTIMATE FATE OF THE BLOOD PLATELETS

It has been shown by Wright[24] that the platelets are formed from the megakaryocytes of the bone marrow and spleen. Their life is short, probably a few days, as shown by Duke and as suggested by the results of transfusion in our hemophilic patient. The ultimate destiny of the blood platelets has not been studied. We have no knowledge on this point. There must be a constant destruction. A possible explanation is that after their disappearance as fixed elements in the blood the active principle presumably is taken up by the tissues and is present in

24. Wright: Jour. Morphology, 1910, xxi, 263.

tissue juice and known as the thromboplastic substance of the fixed tissues. The active coagulating principle of tissue juice accelerates coagulation in a similar fashion as do the blood platelets. If this theory is correct and if we may assume that the difficulty with hemophilic platelets is that they cannot go into solution or start to make thrombin as readily as normal platelets, one ought to be able to show two things: first, that hemophilic tissue juice acts as efficiently as normal; secondly, that hemophilic platelets in solution are more effective than in suspension, that is, in solution their action is like normal platelets in suspension.

Sahli[9] and Morawitz and Lossen[8] have believed that the hemophilic tissue juice was lacking in thromboplastic activity, but they have given no experimental proof. Gressot[25] is the only one who has studied the thromboplastic action of hemophilic tissue. He showed from a study of hemophilic organs obtained at necropsy that the tissue juice of hemophiliacs is as active as normal. One of us had the opportunity one and one-half years ago to study the thromboplastic activity of hemophilic tissues obtained from a patient with typical hemophilia, who died after having transfusion performed. We found, as did Gressot, that we could not detect any ineffectiveness of the hemophilic tissue to accelerate the coagulation of normal blood or normal recalcified oxalated plasma, but its action on hemophilic blood or plasma was not tested. However, the hemophilic tissue acted in no wise differently from normal tissue juice.

On the theory that platelets on being destroyed go into solution and this substance in solution is what is known as tissue juice we might explain the reason for a normal bleeding time in hemophilia in the following manner: Once that the resistant hemophilic platelets go into solution we have an essentially normal tissue juice. It is the tissue juice that is the important element that affects the bleeding time, rather than the number of platelets. The thromboplastic substance appears to be normal in hemophilic tissue and perhaps when a small puncture is made in the ear to test the bleeding time enough of the tissue juice exudes to allow the blood to clot rapidly. If the wound is large so that loss of blood is comparatively rapid and great, we can conceive that enough tissue juice is not available to check such a hemorrhage occurring with a blood with such an abnormal coagulation time. We have occasionally observed that with a grossly faulty technic of vein puncture which allowed a liberal addition of tissue juice, that the hemophilic blood clotted in time approaching that of normal blood and that the hemophilic plasma from such a blood approached normal plasma in its behavior. On the contrary, in cases of purpura hemorrhagica with a diminished number of platelets and

25. Gressot: Footnote 1, third reference.

an abnormally long bleeding time, it is possible to explain the long bleeding time by supposing that it is due to a deficiency of the tissue juice, which in turn is conceivably dependent on a constant replenishment from the solution of the platelets in the tissues, rather than that the bleeding time is entirely dependent on the number of platelets. On such a hypothesis the few platelets would be the cause of the diminished amount of tissue juice and this diminished quantity of active tissue juice would be more directly the cause of the prolonged bleeding time than the few platelets per se.

We have no evidence that tissue juice of cases of purpura hemorrhagica is or is not normal. The observation of Minot[26] on the thromboplastic activity of the uterus removed at operation in such a case is perhaps at first sight against such a theory. He observed that this tissue had thromboplastic activity. On this patient, however, transfusion had been performed forty-eight hours before and also directly before the operation, which might well have altered the thromboplastic content of the organ. Again, differences of thromboplastic activity might be less capable of demonstration on normal plasma than on hemophilic. This was not done.

In regard to the solution of the platelets, we investigated the relative fragility of hemophilic and normal platelets and the efficacy of so-called platelet solutions in accelerating coagulation as contrasted with platelet suspensions. Attempts were made to test the fragility of normal and hemophilic platelets against varying strengths of salt solution, as one tests the fragility of the red cells. No differences could be detected, macroscopically, in regard to their solution. This was because normal platelets even in distilled water do not break up as do red cells. They swell and change their morphologic character somewhat, but to the naked eye the mass left after standing several hours in either normal salt solution or water appears the same. However, if the supernatant fluid is removed from the platelets which have been in water for some time and compared to salt solution in which platelets have stood, the water solution clearly has more thromboplastic action than the salt. Further studies on the microscopic appearance of the platelets after standing in different strengths of salt solution are desirable.

Hemophilic platelet solutions and suspensions were made numerous times and tested to see if we could find any difference in their cytozymic action. It was found impossible by simply using water to get complete solution, so that our platelet solutions always contained some platelets in suspension. The protocol in Table 7 shows the results obtained several times when hemophilic platelet suspensions and extracts were added to hemophilic clear plasma.

26. Minot: Am. Jour. Med. Sc., 1916, clii, 48.

In these experiments it did seem as if the hemophilic platelet solution was more efficient than the hemophilic platelet suspension in its ability to clot the hemophilic plasma. If the solution was kept an hour or so before it was used it was sometimes, but not always, more effective than if used directly after it was made up.

One series of experiments was made with extracts and suspensions of normal and hemophilic platelets on accelerating the clotting time of recalcified hemophilic oxalated plasma. Differences between the action of extracts and suspension were very slight. Ofter both acted equally well and such differences as shown when the plasma was studied were not demonstrated.

Attempts were also made to see if extracts of normal and hemophilic platelets were more effective in forming thrombin with serozyme and dilute calcium than suspensions of normal and hemophilic platelets. The results varied. Perhaps more frequently the extracts seemed to be rather better than the suspensions in being able to form thrombin. On the other hand, the reverse result was sometimes obtained. We have at present no satisfactory explanation for the inconsistent results of these experiments.

From the results of our studies on the ability of platelets in solution to act more efficiently than in suspension, it is seen that one set of experiments showed this to be apparently true, while other experiments were inconclusive or negative. It is desirable to study such differences probably by other means, by aid of other substances to cause better solutions, for example, action of minimal doses of roentgen rays, etc. At present one can say that some results are suggestive of differences. This is not entirely in accord with Fonio,[27] who found solutions of platelets less or equally active with their suspensions.

THE PROTHROMBIN TIME

The prothrombin time, the time it takes a solid clot to form from oxalated plasma on recalcification with the optimum amount of calcium, was determined by the method described by Howell[5] and discussed by Minot,[28] and by Minot, Denny and Davis.[21] This time was found abnormally long, as may be seen by referring to Table 8. Such a markedly delayed prothrombin time is characteristic of hemophilic blood, as has been shown by Howell,[5] Addis,[13] Hurwitz and Lucas,[6] Minot, Denny and Davis,[24] and Hess.[7] There was no evidence that the available calcium was at fault in these hemophilic cases, as evidenced by the fact that the optimum amount of calcium needed to recalcify the plasma from the cases and the controls was the same. The in vitro test

27. Fonio: Footnote 1, first reference.
28. Minot: Jour. Med. Research, 1916, xxxiii, 503.

of Lee and Vincent[29] showed that a drop of calcium added to 1 c.c. of blood or plasma did not alter the coagulation time or shortened it but very slightly, not giving the striking changes seen in certain cases of jaundice.

THE ANTITHROMBIN

The antithrombin was tested for by a modification of Howell's[5] method and that previously used by Minot, Denny and Davis.[21] This modified method gives less variation of the antithrombin in normal blood than that previously found by Minot, Denny and Davis. The antithrombin factor or quotient was found to be normal or more usually slightly above normal (Table 8). Such findings have been reported before. It is unfortunate that we were unable to determine the prothrombin time and antithrombin in the second case after transfusion. A heavy suspension of hemophilic platelets was able to neutralize antithrombin from hemophilic serozymes, plasma or serum as rapidly and readily as normal platelets. Dealing with dilute suspensions of platelets, which are notoriously uncertain in regard to their equality, we seemed to find the hemophilic platelets less able to neutralize antithrombin than were normal platelets.

MISCELLANEOUS STUDIES

The addition of normal or hemophilic active serozyme to whole hemophilic blood or normal or hemophilic recalcified oxalated plasma did not affect the clotting time if the serozyme was free of thrombin.

The addition of an amount of pure thrombin made according to Howell's[30] methods fully sufficient to clot solidly normal blood or oxalated plasma in two minutes likewise clotted solidly in two minutes hemophilic blood, plasma or oxalated plasma. When an amount of pure thrombin was used that just clotted firmly normal plasma the hemophilic plasma might be clotted a little less firmly. Such differences as this in these cases were very slight. In the study of various cases in the past, though not without exception, such a difference has been concomitant with an increase of antithrombin, as it was in these cases.

The addition of cephalin, or tissue extracts, like normal platelets, clotted hemophilic plasma or blood rapidly.

Hemophilic serum clotted normal or hemophilic plasma equally well and in essentially the same time as did normal serum.

We have tested the hydrogen ion concentration of the blood in these cases by the method described by Levy, Rowntree and Marriott[31] and found it normal, as did Hurwitz and Lucas.[6]

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29. Lee and Vincent: THE ARCHIVES INT. MED., 1915, xvi. 59.
30. Howell: Am. Jour. Physiol., 1910, xxvi, 453; ibid., 1913, xxxii, 264.
31. Levy, Rowntree and Marriott: THE ARCHIVES INT. MED., 1915, xvi, 389.

We have found no evidence in several cases of hemophilia of any fibrinolytic ferment present in the circulating blood during life. This is in accord with Morawitz and Lossen[5] and the recent findings of Hurwitz and Lucas.[6]

Dr. A. H. Rowe tested the albumin and globulin content of the plasma by T. B. Robertson's methods and found it essentially normal as did Hurwitz and Lucas.[6]

In regard to the occurrence of nontypical types of hemophilia, we would like to emphasize that there are various atypical and intermediate types of hemophilia and purpura hemorrhagica; that there is undoubtedly a hereditary type of purpura and that this may occur in a female member of a family, some of whose male members are typical hemophiliacs; that atypical cases giving a history of hemophilia may at certain times have a normal or nearly normal coagulation time; that an antithrombin increase may perhaps at times be more marked than normal in some of these cases; that some cases may appear at one time to be more of a hemophilic nature and at other times more of a purpuric nature; that is, at one time they may show a normal platelet count, but a marked delay of the coagulation time, while at other times the coagulation time may be less delayed, while the blood platelets are very few in numbers.

The fact that cases of purpura hemorrhagica are associated with few platelets, on which the bleeding time depends indirectly or directly, and that cases of intermediate types of purpura and hemophilia exists, suggests again that an alteration in the platelets is the cause of the abnormal coagulation phenomenon of hemophilic blood. We would suggest from our studies that the hemophilic platelets are qualitatively defective in that they cannot start the process of coagulation as well as normal platelets, that as cytozyme they cannot act as rapidly to form thrombin or to accelerate the coagulation of the blood. In Howell's terminology we can interpret our findings to indicate that the hemophilic platelets cannot give up their prothrombin so readily as normal platelets, rather than that they are deficient in their prothrombin content; this would agree with Addis' conception of the cause of the slow coagulation of hemophilic blood. If this is true, one would expect to find a marked diminution compared to normal in the circulating prothrombin in solution, while the total prothrombin (in solution and bound) would occur in normal amounts. Perhaps the much delayed prothrombin time of hemophilic plasma is explained on such a basis, that is, that it contains minimal amounts of free soluble prothrombin or its antecedents.

SUMMARY AND CONCLUSIONS

The blood platelets from two typical cases of hemophilia have been studied, because in the course of some work on coagulation we have been greatly impressed by the importance of the blood platelets. Previous work has shown that in typical hemophilia the formed elements are in essentially normal numbers. The calcium and fibrinogen content of the blood and thrombin in the serum are within normal limits. The antithrombin is normal or often slightly increased. The activity of the tissue juice is probably normal. The prothrombin time is markedly delayed. These results agree with the findings in our cases.

The hemophilic blood platelets were obtained directly from the blood and from various types of salted plasmas. If normal blood platelets in about normal amounts were added to hemophilic plasma they caused it to coagulate in a time that is normal or nearly normal. When hemophilic blood platelets were added even in approximately seventy-five times as great a concentration as in normal blood, though they definitely shortened the coagulation time, they never brought that time to anything approaching normal limits.

By using the method of formation of thrombin described by Bordet and Delange, the blood platelets required more time to form thrombin when derived from hemophilic than from normal blood. This is consistent with the reclotting phenomenon observed in hemophilic bloods.

Microscopically, under favorable conditions of thrombin, etc., hemophilic platelets undergo the usual transformation in apparently normal time. Under the most favorable conditions hemophilic platelets act nearly normally. On the other hand, in the case of oxalated plasma, recalcified by an amount of calcium that is not the optimum amount, wide discrepancies are seen in the clotting times when normal and hemophilic platelets are added.

This evidence suggests, as does the fact that partial solution of the hemophilic platelets in water was usually more efficient than hemophilic platelets in suspension, that the delay in coagulation in hemophilia occurs in the initial step in coagulation, which seems to be a rendering of the platelets available by some process like solution.

We are inclined to present the theory that the active coagulating principle of the tissue juice is derived in part, if not wholly, from the blood platelets. As evidence on this point we present the fact that in hemophilia with a normal number of abnormally resistant platelets we have a very abnormal coagulation time, but a normal bleeding time. In purpura hemorrhagica these conditions are just the opposite. The normal number of platelets, though few in number, are sufficient to form a little thrombin and clot fibrinogen in essentially normal time. The value of an excess of platelets seems to be to furnish the active coagulating principle of the tissue juice.

On one of the hemophilic patients transfusion was performed with 600 c.c. of normal blood. The coagulation time before transfusion was from sixty to 120 minutes. After transfusion it was seven minutes. A gradual lengthening of the coagulation time occurred for three days, when it was again sixty minutes. Since about three days is generally assumed to be the length of life of the blood platelet, our actual clinical findings seem to corroborate the findings in vitro.

We conclude that in hemophilia we have an hereditary defect in the blood platelets. This defect consists of a slow availability of the platelets for the purposes of coagulation.

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THE EXCRETION OF CHLORIDS AND WATER AND THE RENAL FUNCTION IN SERUM DISEASE*

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Since the classical work of von Pirquet and Schick on serum disease, it has been repeatedly observed that with the edema which appears in the course of this illness, albumin and occasionally casts may occur in the urine, and a few observers have noted suppression of urine with subsequent albuminuria and cylindruria during severe immediate or accelerated reactions following a second dose of serum. Though these symptoms are said to be transient, no accurate studies have so far been made to determine whether actual changes take place at this time in the functional activity of the kidneys, and since it has been shown by one of us[1] that repeated injections of foreign protein in sensitized animals may lead to damage of the kidney, as well as to the other organs, it seemed important to determine whether the edema, which is often general in serum disease, is associated with any transient impairment of the kidney function.

For this purpose ten cases of serum disease have been studied. Most of the patients were young adults, five below the age of 25; of the remainder, one was 29, one 31, one 36, one 48 and one 49. None of them gave a history which would suggest any previous disease of the cardiovascular system or of the kidneys and none presented any signs of chronic diseases when they entered the hospital. In nine patients serum disease followed the intravenous use of antipneumococcus serum and in one the intraspinal injection of antimeningococcus serum.

The methods employed to study the functional activity of the kidneys have been as follows: Immediately after the patient received the first therapeutic injection of serum, which in every instance was horse serum, he was given a suitable diet that contained as nearly as possible a constant quantity of sodium chlorid. This varied in different instances, but during the period of the disease in which the diet contained fluid or only eggs, bread and cereals, the patient received from 2 to 3 gm. of salt a day; later when the diet was increased the amount of sodium chlorid varied from 6 to 10 gm. a day. The total fluid intake was fixed at 2,500 c.c. or 3,000 c.c., according to the needs of the

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1. Longcope: Jour. Exper. Med., 1913, xviii, 678.

person. On this constant intake of sodium chlorid and fluid the daily output of urine was measured and the chlorid estimated by the Mohr method. In a few instances the chlorids of the plasma have been determined by the McLean and Van Slyke modification of Volhard's[2] method, at various intervals, before, during and after the serum sickness. In the same way the total blood urea has been estimated by the urease method and the index of urea excretion studied according to McLean's modification[3] of the Ambard formula. Together with these procedures the excretion of phenolsulphonephthalein has been tested at intervals during the course of the disease.

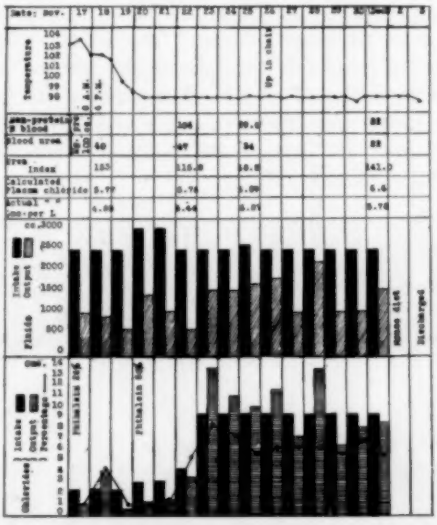

Fig. 1.—M. S., man, aged 34, with lobar pneumonia, pneumococcus Type I. The usual course of water and chlorid excretion, the changes in nonprotein nitrogen, urea and chlorids of the blood and phenolsulphonephthalein excretion during convalescence from uncomplicated lobar pneumonia.

As most of the cases of serum sickness occurred during convalescence from pneumonia and since it is known that the excretion of chlorid as well as the nitrogenous metabolism is greatly disturbed in this disease, it was necessary to apply, as a control, the same methods of study to several convalescing pneumonia patients who had not received serum. Five such patients were followed during their convalescence. During the attack of pneumonia, as is well recognized, the excretion of chlorid is greatly diminished. The plasma chlorids fall

2. Jour. Biol. Chem., 1915, xxi, 361.
3. Jour. Exper. Med., 1915, xxii, 212.

below normal and the amount of urine is small. Three to four days after the crisis the plasma chlorids rapidly rise and the excretion of chlorids increases so that the chlorids retained during the disease are eliminated within a few days. During this time they are excreted in excess of the intake and in high concentration. Very rapidly, however, a balance is established and the normal ratio of excretion is reached.[4]

The curves shown in Figure 1 show the characteristic changes in water and salt excretion of the five cases studied as controls.

During the convalescence from pneumonia there may be a disturbance in nitrogenous metabolism, the total blood urea varying considerably from the normal figure. For this reason no deduction can be drawn from the estimations of urea and the nonprotein nitrogen of the blood in the cases of serum disease. The index of urea excretion, however, remained unaltered in three of the control cases. Table 1 gives the determination of phenolsulphonephthalein for a two-hour period, of the blood urea, the urea index and the plasma chlorids in four control cases. In Cases 1 and 4 these estimations were made both before and after the crisis.

TABLE 1.—PHENOLSULPHONEPHTHALEIN EXCRETION, BLOOD UREA AND INDEX OF UREA EXCRETION AND PLASMA CHLORIDS IN CASES OF UNCOMPLICATED LOBAR PNEUMONIA

Control Cases Uncomplicated Pneumonia	Date	Phenolsulphonephthalein, per Cent.	Blood Urea, Mg. per 100 c.c.	Urea Index	Plasma Chlorids, Gm. per Liter	Remarks
1	10/18	26	40	153	4.88	Before crisis
	10/20	50	..	...	....	Before crisis
	10/22	..	47	115.8	5.44	After crisis
	10/25	..	34	68.5	5.67	After crisis
	12/ 1	..	22	141	5.78	After crisis
2	1/14	64	16	171	5.5	After crisis
3	1/ 4	46	..	...	....	At crisis
4	11/17	56	..	...	....	Before crisis
	11/30	51	..	...	....	After crisis
	12/ 1	..	32	113	5.52	After crisis

As compared with these controls a study of the ten cases of serum sickness showed that during this illness the excretion of phenolsulphonephthalein and urea was little affected. Table 2 gives the estimation of phenolsulphonephthalein for a two-hour period, the blood urea and index of urea excretion in the ten cases. In Case 3 the excretion of phenolsulphonephthalein has slightly diminished, in Case 4 at the

4. Peabody: Jour. Exper. Med., 1913, xvii, 71. McLean: Ibid., 1915, xxii, 366.

onset of serum sickness the phenolsulphonephthalein excretion was far below normal and the blood urea distinctly elevated. Unfortunately the index of excretion was not determined, but before recovery from serum disease the blood urea had fallen to the normal level. In Case 9 the blood urea was also high, but the index normal. In Case 10 there was a transient but marked reduction in excretion of phenolsulphonephthalein during the serum sickness.

Much more profound changes were observed, however, in the excretion of water and chlorids than in the excretion of nitrogen and

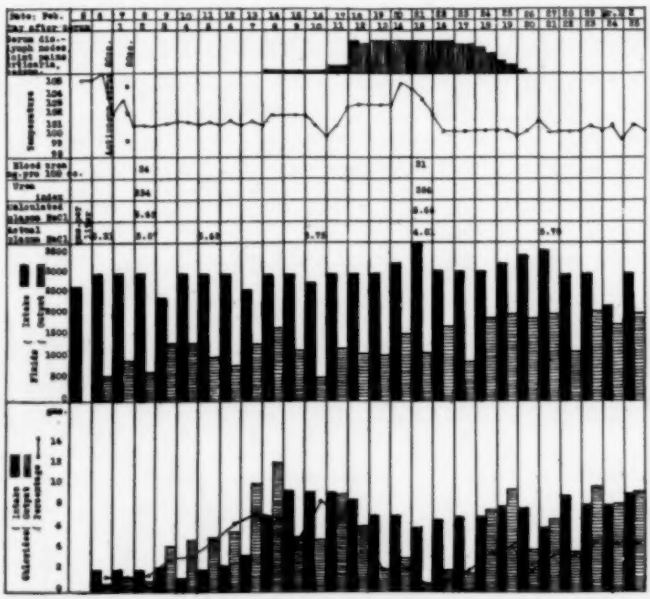

Fig. 2 (Case 5).—W. B., man, aged 31, with lobar pneumonia, pneumococcus Type 1, with serum disease following the intravenous injection of serum. The course of water and chlorid excretion, the changes in blood urea, urea index, plasma chlorids, and excretion of phenolsulphonephthalein during convalescence from pneumonia complicated by serum disease. The severity of the serum disease is indicated by the height of the block in that section.

phenolsulphonephthalein. This was observed in eight of the ten cases. The two cases that did not show any deviation from the controls were Cases 1 and 2.

In Case 1 the patient received two doses of 90 c.c. of antipneumococcus serum intravenously, which was followed in eight days by mild urticaria lasting forty-eight hours, while in Case 2 the administration of the same amount of serum intravenously was followed in eight days by a fairly severe urticaria of four days' duration.

TABLE 2.— PHENOLSULPHONEPHTHALEIN EXCRETION, BLOOD UREA AND INDEX OF UREA EXCRETION AND PLASMA CHLORIDS BEFORE, DURING AND FOLLOWING SERUM DISEASE

Case No.	Before Serum Disease				During Serum Disease				After Serum Disease			
	Phenol-sulphone-phthalein, per Cent.	Blood Urea, Mg.	Urea Index	Plasma Chlorids	Phenol-sulphone-phthalein, per Cent.	Blood Urea, Mg.	Urea Index	Plasma Chlorids	Phenol-sulphone-phthalein, per Cent.	Blood Urea, Mg.	Urea Index	Plasma Chlorids
1	48	32	480	....	58	24*	254	....	..	22	436	....
	..	..	...	....	..	24	410	....	..	18	466	....
2	30	45	...	....	54	31	...	....	..	26	...	....
	59	36	...	....	..	22	...	....	..	..	...	....
3	..	..	...	....	45	32	...	....	60	28	...	....
	..	..	...	....	33	..	...	....	..	..	...	....
	..	..	...	....	47	..	...	....	..	..	...	....
4	..	..	...	....	16.4	110	...	....	..	..	...	....
	..	..	...	....	48	24	...	....	..	..	...	....
	..	..	...	....	..	20	...	....	..	..	...	....
	..	..	...	....	45	20	...	....	..	..	...	....
5	75	34	234	5.31	64	31	386	4.81	..	..	...	5.78
	..	..	..	5.87	..	..	...	....	..	..	...	....
	..	..	...	5.62	..	..	...	....	..	..	...	....
	..	..	...	5.75	..	..	...	....	..	..	...	....
6	..	..	...	....	80	24	...	....	..	..	...	....
	..	..	...	....	..	31	...	....	..	..	...	....
7	54	16	300	6.125	58	11	148	6.50	62	10	235	6.56
8	..	29	...	....	50	22	...	....	..	..	...	....
	..	..	...	....	62	29	...	....	..	..	...	....
	..	..	...	....	..	16	...	....	..	..	...	....
9	56	..	...	....	66	46	126	....	64	12	162	....
	..	..	...	....	..	65	191	....	74	24	133.75	....
10	50	40	111	....	18	20	113	....	65	..	...	....

* When two or more figures occur in the same column for a single case, they represent separate estimations at from two to four days interval.

The remaining eight cases fall into two distinct groups. In the first, which comprises six cases (Cases 3, 4, 5, 6, 8 and 9) the following changes were observed: At the onset of serum sickness the excretion of chlorids fell to a low figure, so that on an intake of 5 gm. of sodium chlorid, less than 1 gm. was eliminated. At the same time the total volume of urine diminished so that the chlorids were excreted in very low concentration. During this time the addition of 10 extra grams of sodium chlorid to the diet in Cases 4 and 8 (Fig. 3) caused no increase in excretion of chlorids in the urine. With recovery from the symptoms of serum sickness the chlorids were eliminated greatly in excess

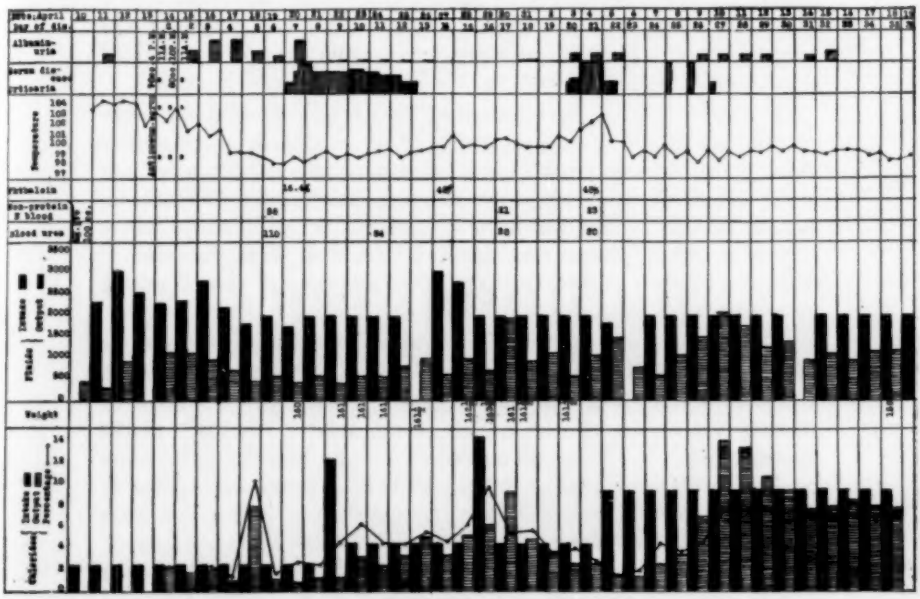

Fig. 3 (Case 4).—M. H., woman, aged 49, with lobar pneumonia, pneumococcus Type 1, with serum disease following the intravenous injection of serum. The course of water and chlorid excretion, the changes in the nonprotein nitrogen of the blood, blood urea, and excretion of phenolsulphonephthalein, in convalescence from pneumonia complicated by severe and relapsing serum disease. The severity of the serum sickness is represented by the height of the blocks in that section.

of the intake, in moderately high concentration, and were often associated with a mild diuresis. One patient, Case 8, who on an average intake of 4.5 gm. of sodium chlorid had been retaining chlorids during a prolonged attack of serum disease lasting twenty-two days, eliminated after recovery 12 to 16 gm. of chlorids on a daily intake of 6 gm. of sodium chlorid, while the daily output of urine on an intake of 2,500 c.c. of fluid suddenly increased about 1,000 c.c.

The degree of water and chlorid retention seems to depend on the intensity of the serum disease, rather than on the amount of serum given, for the disturbances were quite as marked in one boy of 15 who received two doses of 40 c.c. of antipneumococcus serum intraspinally as in a man of 31 who received a total of 160 c.c. of serum intravenously.

Figures 2 and 3, from Cases 5 and 4, illustrate well the type of alteration observed. In Case 5 (Fig. 2), that of a man of 31, the critical close of his pneumonia was followed by the usual excessive excretion of chlorids in high concentration. With the onset of serum disease the daily elimination of chlorid immediately fell from 1 to 3 gm. a day on an intake of from 6 to 7 gm., and the percentage was reduced below 0.2. With recovery from serum disease the retained chlorids were again excreted and the quantity of urine increased.

In Case 4 (Fig. 3) the injection of 240 c.c. of serum in four doses resulted in a severe and relapsing type of serum disease lasting twenty days. It will be seen from Figure 3 that the additional 10 gm. of salt in the diet on the third day of the serum disease was not excreted and that the same procedure during the intermission between the two severe attacks of serum disease was followed by a very slight response, as evidenced by the moderate increase in the excretion of chlorid during the next forty-eight hours.

The question immediately arises as to whether the chlorids in these cases are retained in the tissues or in the blood. McLean has reported a case of serum disease after pneumonia in which the retention of chlorids was associated with a high plasma chlorid. A study of the plasma chlorids in this group seemed to indicate that the chlorids are retained in the tissues rather than in the blood. In one case (Case 5) the plasma chlorids were carefully followed before, during and after the serum sickness. The results are shown in Table 2 and in Figure 2. Immediately before the crisis the plasma chlorids were somewhat lower than is generally conceded to be the normal figure for the threshold of excretion, which is put at 5.62 gm. per liter. At this time the plasma chlorids were 5.31 gm. per liter. Immediately after the crisis they rose to 5.87 gm. per liter; three days later they were 5.62 gm. per liter. The day before serum disease appeared they were 5.75 gm. On February 21, the fourth day of the serum disease, they had fallen to the extremely low figure of 4.81 gm., while on February 27, two days after recovery from serum sickness, the plasma chlorids had risen to 5.78 gm. and chlorids were being excreted in large quantities in the urine.

We may therefore conclude that in this group of cases the marked retention of water and chlorids takes place in the tissues and is associated regularly with albuminuria of mild degree, with the presence of

casts in the urine and occasionally (Cases 3 and 4) with a transient disturbance in the excretion of phenolsulphonephthalein.

In the second group, which comprises only two cases, 7 and 10, the excretion of salt and water is affected in a different manner. Both of these patients were women, one 48 years of age, the other 29 years old. One patient (Case 7) received three intravenous injections of 80 c.c. of serum, which was followed on the seventh day by mild serum disease, consisting of urticaria and enlargement of lymph nodes, but which persisted for ten days. There was no excessive retention of

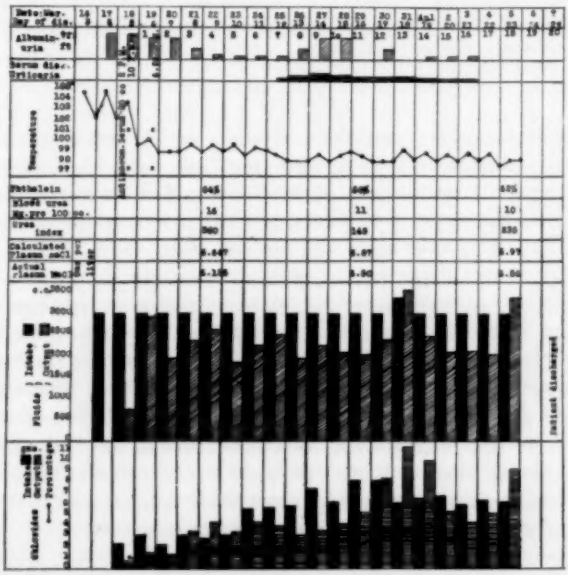

Fig. 4.—E. F., woman, aged 48, with pneumonia, pneumococcus Type 1, with serum disease following the intravenous injection of serum. The course of water and chlorid excretion, the changes in blood urea, the urea index, the plasma chlorids and phenolsulphonephthalein excretion during convalescence from pneumonia complicated by serum disease. The severity of the serum disease is represented by the height of the blocks in that section.

chlorids or water, but the patient was incapable of concentrating the chlorids in the urine and excreted fair quantities of chlorids in low concentration. This is well illustrated in Figure 4. It will be seen that on two occasions, March 31 and April 5, when chlorids were excreted in excess of the intake, an actual diuresis occurred.

In Case 10 the same type of excretion was observed. In both instances there were albuminuria and cylindruria, which disappeared after the subsidence of the serum disease. In this group, therefore,

the serum disease was accompanied by a hyposthenuria. A study of the blood chlorids in Case 7 showed that they were always high before, during and after the attack of serum disease, but tended to rise during the serum disease from 6.25 gm. per liter to 6.5 gm. per liter. In this group, therefore, it is evident that there is a retention of chlorids in the blood, though it is impossible to exclude the tissues as well. They are, however, excreted through the kidneys only in dilute solution, which results in what Schleyer has termed hyposthenuria. It is possible that in these two instances there was some previous injury to the kidney which resulted in this type of excretion. But so far as could be determined from subsequent study of one of these patients, these alterations were not persistent.

COMMENT

These studies show very definitely that during serum disease there is often a marked but transient retention of chlorids and of water, associated frequently with slight albuminuria and cylindruria and occasionally with impairment of the excretion of phenolsulphonephthalein. In five of the ten cases there was obvious edema with gain in weight. In the other cases there was no edema and no gain in weight. In the first group it is probable, since the plasma chlorids were below normal, that some disturbance occurred primarily in the tissues during the intoxication, which cause a retention of salt and water in this situation, and was associated with a generalized edema. There may also have been some functional impairment in the excretory action of the kidneys. In the second group, in which the plasma chlorids were high, the disturbance consisted principally in the impaired elimination of chlorids and the inability to concentrate these organic salts in the urine. In the two cases in this group edema was not observed.

INFLUENCE OF AGE AND SEX ON HEMOGLOBIN

A SPECTROPHOTOMETRIC ANALYSIS OF NINE HUNDRED AND NINETEEN CASES *

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To the thoughtful clinician it has long been a matter of keen regret that the very examination of the blood which is perhaps most frequently made in routine practice, namely, the estimation of the percentage of hemoglobin, is the one from which we generally derive the most unsatisfactory results. Aside from the very glaring defects in some of the instruments in common use, this has been due to the fact that we have had few accurate data as to the variations in the amount of hemoglobin at different ages. What knowledge we possess on the subject dates from the work of Leichtenstern.[1] This author recognized very clearly that before any conclusions could be drawn as to whether a person of a given age and sex had a normal amount of hemoglobin, it was first necessary to determine the norm for that age and sex. In addition to studying the effects of a number of other physiologic conditions on the hemoglobin content of the blood, Leichtenstern made determinations of the hemoglobin in a number of normal persons of both sexes, and at various ages, in order to determine the variations due to age and sex. Practically all of the knowledge of these factors which we possess is derived from this work. There are, however, a number of reasons why Leichtenstern's work is inadequate. In the first place, at that time it was not possible to crystallize human oxyhemoglobin, and therefore his results were only relative. Any absolute figures attributed to him have been obtained by using the extinction coefficients given by him with a factor obtained years after by others. Since, as was shown later by Butterfield, this factor may vary materially with slightly different conditions of experiment, and especially with different instruments, the propriety of this is more than questionable.

In addition, his observations were made on too few persons to give anything more than approximate results, a fact which he himself plainly states. For example, he examined no infants on the first day of life, and his values for infants up to four days are based on only four determinations, three in boys and one in a girl, the youngest being

* Submitted for publication June 13, 1916.

1. Leichtenstern: Untersuchungen über den Haemoglobingehalt des Blutes in gesunden und kranken Zuständen, 1878.

only 36 hours old. For three age periods (from 51 to 56, from 56 to 60 and over 60) he based his figures on a total of only five cases, for both men and women, so that in these instances the figures for one sex are based on the examination of only two persons. Such results are, of course, entirely unreliable. He did not carry his age periods beyond the sixtieth year.

In view of the inadequacy of Leichtenstern's work, and in view of the absence of any extended investigations, with methods of precision, bearing on these points, it seemed very necessary that the entire matter should be reinvestigated on an amply large material, and employing a method of acknowledged and demonstrable accuracy.

The fact that Leichtenstern's work deals entirely with persons living under conditions as they then existed in Germany, and who might or might not present hemoglobin concentrations identical with those of our patients in America, seemed a further reason for undertaking this research.

METHOD OF STUDY

Of all the methods for the estimation of hemoglobin which have been proposed, the spectrophotometric is unquestionably the most accurate. In addition to its great accuracy it possesses the important advantage of requiring only a small amount of blood, and the technic of using the spectrophotometer, when once acquired, is relatively simple, although somewhat time-consuming. Even in the comparatively primitive form, as used by Vierordt, it was a very exact instrument, and in the improved form, as used by Huefner, it is even more so. Our instrument was a new Huefner apparatus, as made by Albrecht of Tübingen.

Some months of time were devoted to attaining the necessary familiarity with the instrument so as to insure a sufficiently high degree of accuracy. A critical study of the possible sources of error was made, which resulted in the development of a technic which could be depended on to give results of a marked degree of uniformity and exactness. Through the kindness of Prof. George Dreyer, head of the department of physiology, the facilities of the physiologic laboratory were placed at our disposal. In addition, it is a pleasure to acknowledge our indebtedness to him for much advice and assistance during this preliminary work, which was begun early in 1914.

QUANTITATIVE SPECTROPHOTOMETRIC DETERMINATION OF HEMOGLOBIN: PRINCIPLES INVOLVED

Without attempting to go into the general subject of spectrophotometric examinations, the principles of which must be assumed to be already known, it may be in place to give briefly the salient points. For each colored substance the ratio, concentration to extinction

coefficient, is a constant. This constant is known as the absorption ratio. This law is generally expressed $C/E=A$. The concentration C in the case of blood is the number of grams of oxyhemoglobin in 1 c.c. of the solution examined. The extinction coefficient is the reciprocal of the thickness of solution required to reduce light of the intensity one to the intensity one tenth. In the Huefner apparatus the thickness of the layer of solution remains constant, and E is determined by measuring the intensity of the remaining light. This is effected by rotation of the analyzing with respect to the polarizing Nicol. Now it can be readily shown that the extinction coefficient, E, is equal to -2 log. cos. O, where O is the angle of rotation just referred to. In order to obtain E we measure the angle of rotation, O, necessary to produce equality in the upper and lower fields. This angle is read off on a vernier, and from this the extinction coefficient, E, is computed. Buerker has computed a curve of extinction coefficients for angles from 60 to 82 degrees, which greatly facilitates this computation. For solutions of one and the same substance, in our case oxyhemoglobin, the concentrations are directly proportional to their respective extinction coefficients, so that these are used as a convenient method of expressing relative concentrations. To convert relative into absolute values a solution of oxyhemoglobin of known strength must be prepared, its extinction coefficient, E, determined, and then $A=C/E$, or $C=E\times A$. In other words, the extinction coefficient of any solution multiplied by the absorption ratio thus obtained gives the absolute concentration of the solution. This absorption ratio was determined by Huefner for oxyhemoglobin for the spectral region from 531.5 to 542.5, and found to be 0.001312. Butterfield[2] found it to be 1.18×10^{-3} for the spectral region from 533.5 to 542.

The exact position in the spectrum, the width of slit and several other factors exercise a great influence on the value of A, as Butterfield has shown, so that it is essential, in order to be sure of the results, for each investigator to determine the value of A for his exact conditions of experiment. Since the publication of the preliminary report of this research we have done this, and the absolute values, as given in this paper, represent the relative values previously given multiplied by the factor A, with, of course, the proper correction for dilution. It is unnecessary to describe the Huefner apparatus. We had a special dark room at our disposal and the entire apparatus was further enclosed in a large hinged light-proof case, so constructed that when in use no extraneous light could reach the eye. This is a matter of much impor-

2. Butterfield, E. E.: Ueber die Lichtextinktion, das Gasbindungsvermögen und den Eisengehalt des menschlichen Bludfarbstoffs in normalen und krankhaften Zuständen, Ztschr. f. physiol. Chem., 1909, lxii, 173.

tance, since eye fatigue is the principal source of error in the method. The sector scale was calibrated in the usual manner, so that the divisions on it could be converted into wave lengths. To assure ourselves that the positions actually chosen really represented the positions of maximum and minimum absorption we determined the degree of absorption for the entire area. The positions found were from 542 to 534 and from 566.5 to 558.5. Our collimator slit was set at 0.05 mm., and remained unchanged throughout all the work. The eye piece slit was set at 8.

DETERMINATION OF THE EXTINCTION-RATIO (E'_o/E_o)

It is well known that Vierordt and his school have, through a long series of investigations, enunciated the law on which the qualitative spectrophotometric determination of hemoglobin, as well as its derivatives, is based. If E' be the extinction coefficient in one part of the spectrum, and E be the extinction coefficient in the other part of the same spectrum, then the extinction ratio E'/E is constant for one and the same hemoglobin derivatives, even at different concentrations; while on the other hand, the ratio E'/E is different for each derivative and characteristic of it. For oxyhemoglobin the ratio $E'o/Eo$ has been found by Huefner to be 1.578, or as it is generally expressed in round figures, 1.58.

Bohr[3] has called into question the identity of hemoglobin from different sources and considers that there are several hemoglobins. His researches do not bear out the contention of Huefner that the ratio $E'o/Eo$ is one and the same under identical conditions of experiment.

Aron and Müller[4] in 1906 also found an inconstancy of this ratio. On the other hand, Butterfield, using the Huefner apparatus, conducted an extensive series of experiments, and came to the conclusion that the light extinction, iron content and oxygen-binding capacity of human hemoglobin are, within the limits of error of the method employed, constants. He further found that these constant values applied, not only to the blood of normal human beings, but also to those bloods derived from patients with anemia, chlorosis, scorbutus and pseudoleukemia. While it was not the principal purpose of this research to determine the constancy of this coefficient, yet inasmuch as the value of the spectrophotometric qualitative analysis depends on the extinction ratio, it was felt to be highly desirable to determine this constant anew under the conditions of our experiments, in spite of the

3. Bohr: Nagel's Handbuch der Physiologie des Menschen, i, 51 (Bibliography).

4. Aron and Müller: Ueber die Lichtabsorption des Blutfarbstoffs, Arch. f. Physiol., 1906, Suppl., p. 109; Ztschr. f. physiol. Chem., 1906, lvi, 443.

apparently conclusive work of Butterfield. To this end, in each and every observation, readings were taken not only in the position of maximum, but in the position of minimum absorption, and from these values the extinction ratio, that is, *E'o/Eo,* was calculated for each determination. Because of the large number of determinations made by us, it was felt that the average of all of these would give a very close approximation to the real value. Every observation is included except those in which the reading in the region between the bands was below 60 degrees, since we, in common with all observers, found the accuracy in reading diminishes rapidly below this point. These observations numbered in all 838, and the ratio was found to be perfectly constant within the limits of error of the method, and to average 1.5813. Our results, therefore, corroborate in fullest measure those of Huefner and Butterfield, not only in regard to the constancy of the extinction ratio, but also in regard to its actual value. Huefner's[5] determinations average 1.578, as against our value of 1.5813.

CONSTRUCTION AND CALIBRATION OF PIPET

It is obvious, in view of the high degree of accuracy attained in the actual reading of the spectrophotometer, that the error involved in measuring and diluting the blood is likely to be several times as great as the error of reading. Leichtenstern recognized this, and came to the conclusion that it might be ten times as great. Our preliminary work led to the same conclusions, and to reduce the error in obtaining and diluting the blood to a minimum, considerable experimentation and thought were given to the question of a suitable pipet. In this matter we were rendered invaluable aid by Professor Welker, to whose kindness we are indebted for the construction and calibration of the pipet and to whose advice and skill the accuracy of this part of the work is entirely due.

In its final form the pipet consisted of a piece of heavy thermometer tubing with thick walls, but of fine caliber, and yet sufficiently large that when it was filled with blood and held vertically the column would flow down with that degree of rapidity best suited for accurate measurement. It was approximately 7 inches in length, with a circular mark about 4 inches from the tip, the latter being drawn out so as to constrict the opening to the least possible extent. Two separate calibrations of the pipet were made, with defibrinated blood, in the most careful manner, and the average of the two determinations gave the weight of blood delivered by it as 0.03865.

5. Huefner, G.: Neue Versuche zur Bestimmung der Sauerstoffkapazität des Blutfarbstoffs, Arch. f. Physiologie, 1894, p. 130.

The specific gravity of the blood was determined with two separate pycnometers, each of a different type, and found to be 1.0601 and 1.0597 respectively, the average being 1.0599. The volume of blood delivered by the pipet was, therefore, $0.03865/1.0599 = 0.03646$ c.c. It was found that this amount could be readily obtained from either the finger in adults or ear in children without resorting to undue manipulation. The advantages of having the pipet of a larger capacity than those in common use are obvious.

TABLE 1.—Comparison of Two Dilutions to Determine Accuracy of Pipet

Solution A		Solution B	
77.9	77.8	77.4	78.0
77.3	77.5	78.0	78.0
77.9	77.6	77.5	77.4
77.4	77.7	77.4	77.6
77.8	77.9	78.0	77.5
77.6	77.6	77.6	77.4
77.9	78.0	77.4	77.4
77.4	77.4	77.4	77.7
78.0	77.6	77.4	77.9
77.4	77.5	77.4	77.6
776.6	776.6	775.5	776.5

A further check of the accuracy of the calibration was made in the following way. A pipetful of defibrinated blood was added to 4 c.c. of 0.1 per cent. sodium carbonate solution, giving a dilution of 1 in 110.709. Similarly 1 c.c. of the same blood was added to 109.709 c.c. of sodium carbonate solution, and these two dilutions were then compared spectrophotometrically. The result of the comparison is shown in Table 1.

The averages were 77.66 and 77.60, respectively with the two solutions, and the extinction coefficients were 1.340 and 1.336 respectively.

The concentrations of the two specimens, as measured by these two methods, are proportional to their extinction coefficients, that is, the dilutions are to each other as 1.340 to 1.336, which gives further evidence of the accuracy of the pipet calibration. One and the same pipet was used throughout all the work. All dilutions were carried on in precisely the same manner, using a Mohr normal 5 c.c. pipet for that purpose. In the comparison given a Mohr 1 c.c. normal pipet and a normal buret were used, all bearing the standardization mark of the German Reichsanstalt.

METHOD OF OBTAINING AND DILUTING THE BLOOD

Considerable preliminary experimentation convinced us that the most satisfactory source of blood, when uniformity is the great desideratum, is the finger pulp. We used in all cases the middle finger of the left hand, except in young children, in whom the lobe of the ear was chosen. The manner of making an incision is of importance. We used cataract knives of the best quality, which were sent to the maker at short intervals for sharpening. A deep stick gave a very large flowing drop of blood, from which the pipet was filled to a point just a trifle above the mark, and then with the pipet held vertically the column was allowed to sink down exactly to the mark, the excess of blood on the tip being removed by the thumb and forefinger. This blood was then blown into 4 c.c. of 0.1 per cent. sodium carbonate solution, which had been previously measured with a Mohr 5 c.c. normal pipet into a 50 c.c. long-necked, glass-stoppered flask. Our pipet was graduated as a delivery pipet, and by blowing with force each time for ten seconds and withdrawing the tip of the pipet from the fluid, while still blowing, a high degree of uniformity was secured, as will be seen from the accuracy determinations below. The solution must be absolutely clear.

The diluted blood was then thoroughly shaken to ensure complete oxygenation, which is considerably facilitated by the relatively large size of the flask. The blood solution was now transferred into the *Trögchen* of the apparatus, which was covered with a glass slip to exclude atmospheric influences, and ten readings were taken, five on each side of the vernier scale, in the selected position (542.0 to 534.0). In all cases readings were also taken in that position of the spectrum lying between the bands (566.5 to 558.5), so that the extinction ratio could be instantly calculated, and any possible deterioration in the specimen thus disclosed. We regard this as an indispensable precaution, since it obviates the possibility of any error due to a reduction of the oxyhemoglobin, or to a possible partial conversion into methemoglobin.

SOURCES OF ERROR

In any research having to do with the establishing of norms, it is highly important to be orientated as to the possible sources of error and the magnitude of these. In our work we must consider the following: (1) error in the calibration of the pipet; (2) error in taking and diluting the blood; (3) error due to a possible reduction of the oxyhemoglobin to reduced hemoglobin or methemoglobin; and (4) error in reading.

1. *Error in Calibration.*—Any error in this respect is of course a constant one. As stated above, the volume of our pipet was obtained by

taking the mean of two calibrations, agreeing closely with each other. In addition this was checked, as above stated, by comparing spectrophotometrically the dilution, as made by our pipet, and a second similar dilution made with normal pipets standardized by the German Reichsanstalt. Two dilutions made in this way gave extinction coefficients, as we have already seen, of 1.340 and 1.336, respectively. The close agreement of these figures, in addition to the trifling variations of the figures of the two standardizations, makes it sufficiently obvious that the error of calibration is an entirely negligible factor. Since any error would be constant, it could have no effect on the relative values.

2. *Error in Taking and Diluting the Blood.*—This is, of course, a variable error. In the calibration of our pipet we proceeded in the following manner: A weighing bottle, with a roll of filter paper, was first weighed, and after the pipet had been filled with blood in the usual manner, this was blown into the bottle, the excess being wiped off on the paper and contents weighed again. Two determinations were thus made, and the weights obtained may be used as an index of the error involved in measuring the blood. They were 0.0385 and 0.0388.

Our pipet was therefore of such construction as to admit of exceedingly accurate measurements.

3. *Error Due to Deterioration of the Oxyhemoglobin.*—As we have already indicated, by taking readings in the positions of both maximum and minimum absorption, we could at once calculate the extinction ratio $E'o/Eo$ for each observation, so that no appreciable deterioration could occur without being detected immediately. All of our readings were made shortly after taking the blood, so that any deterioration was a priori, improbable. Further to test the degree of deterioration dilutions were made in the usual manner, and tested spectrophotometrically, the first immediately, the second at the end of one hour, and the third at the end of two hours. The extinction coefficients thus obtained were (immediately) 1.300, (one hour) 1.290, (two hours) 1.297. This check was made repeatedly, and in no instance did we find a deterioration sufficient to be detected, within two hours' time.

4. *Error in Reading.*—The construction of the Huefner apparatus is such that the readings can be taken with great accuracy after sufficient practice. This will be apparent from a mere inspection of the readings above given. The Gauss method, which Leichtenstern,[1] Reinert[6] and Buerker[7] have employed, enables us to determine the average and probable errors.

6. Reinert: Die Zaehlung der Blutkoerperchen, Leipzig, 1891.
7. Buerker: Tigerstedt's Handbuch, Vol. 2.

Taking a typical example, we find in one accuracy determination (Jan 1, 1915) the readings given in Table 2.

TABLE 2.—READINGS IN AN ACCURACY TEST OF HUEFNER APPARATUS *

Number Readings	Square of Variation	Variation from Average	Reading Left	Reading Right	Variation from Average	Square of Variation
1	0.0289	+0.17	76.6	76.6	+0.17	0.0289
2	0.0529	−0.23	76.2	76.4	−0.03	0.0009
3	0.0049	+0.07	76.5	76.5	+0.07	0.0049
4	0.0169	−0.13	76.3	76.6	+0.17	0.0289
5	0.1849	−0.43	76.0	76.6	+0.17	0.0289
	0.2885	.....	....	....	.....	0.0925

* Average of ten readings 76.43. $Sd^2 = 0.3810$.

Let d equal the variation from the average.
Let Sd^2 equal the sum of the squares of the individual variations.
Let Fm equal the average error of the average value.
Let N equal the number of observations.
The average error of the average value

$$F_m = \sqrt{\frac{Sd^2}{n\ (n-1)}} = \sqrt{\frac{0.3810}{10\ (10-1)}} = \pm 0.0653$$

The angular error is thus plus or minus 0.0653 degrees.

Now, 76.43 + 0.0653 = 76.4953 = approximately 76.50
76.43 — 0.0653 = 76.3647 = approximately 76.36

Corresponding to these angles we have the extinction coefficients:

$E'o$ of 76.50 = 1.264
$E'o$ of 76.36 = 1.256

If we calculate by the same method the probable error, we find it to be $\pm\ 0.0653 \times 0.6745 = 0.0440$.

These results justify the conclusion that with ten readings the error of the average of these readings is exceedingly small, and can be safely disregarded.

ACCURACY DETERMINATIONS

Before making any of our record observations, we instituted a series of determinations extending over some time to assure ourselves that the necessary amount of familiarity with the method had been secured to attain a high degree of precision. We employed the following method: A pipetful of defibrinated blood was diluted in the usual manner with 4 c.c. of sodium carbonate solution, and the pipet washed and dried. A second pipetful of the same blood was then taken and diluted in the same way. These were then examined spectrophotometrically, ten readings being taken and the same *Trögchen* being used

in both cases. A typical determination of this sort, made on Oct. 30, 1914, is given in Table 3.

TABLE 3.—A Typical Accuracy Test of The Method

Solution A		Solution B	
78.1	77.9	77.8	77.9
78.4	78.5	78.0	78.0
78.2	77.9	78.0	78.2
78.7	78.3	78.6	78.5
78.0	78.0	78.2	77.9
391.4	390.6	390.6	390.5

The averages were 78.20 and 78.11 for the two solutions, respectively, and the extinction coefficients were 1.379 and 1.372 respectively.

To determine the effect of increasing practice on the degree of accuracy, another series of tests were made several months later, some hundreds of determinations having been made in the interim. A typical series is here given in which on Feb. 7, 1915, three successive dilutions were made in the manner above indicated. In dilution No. 1 the extinction coefficient 1.300 was obtained, and in dilutions Nos. 2 and 3, 1.290 and 1.297 were respectively obtained.

It is obvious from these figures that the degree of accuracy attained is almost precisely the same from the beginning of our recorded observations. In order to eliminate the possibility of any self-suggestion in the reading of similar dilutions, we made further tests in the following manner: One pipetful (A) of blood was diluted with 4 c.c. of sodium carbonate solution, a second (B) with 4.5 c.c. of the same fluid. Calculation shows that the concentrations of these two solutions are to each other as 124.42 to 110.71. These two solutions were then examined spectrophotometrically with the results shown in Table 4.

TABLE 4.—Accuracy Test on Different Concentrations of the Sodium Carbonate Solution

Solution A		Solution B	
78.7	78.5	75.6	76.2
78.8	78.7	76.3	75.9
78.4	78.7	76.5	76.2
78.5	78.4	76.5	76.4
78.3	78.6	76.1	76.3
392.7	392.9	381.0	381.0

The averages were 78.56 and 76.20 with the respective solutions, and the extinction coefficients were 1.405 and 1.245.

Taking the ratios, we find $1.405/1.245 = 1.128$,

and $124.42/110.71 = 1.124$.

In other words, the two solutions were so prepared that their concentrations were to each other as 1 to 1.124, while the observed concentrations were as 1 to 1.128.

A considerable number of such tests were made with similar results. Our study of the spectrophotometric method leads us to the conclusion that it is capable of quite an extraordinary degree of accuracy, provided that the investigator have the necessary familiarity with the apparatus. With adequate practice, the error of observations made with the precautions we have used, probably does not exceed 1 per cent. We also made determinations, using thirty readings instead of ten, but were unable to increase the accuracy by so doing. Indeed, there is a real danger of lessening it by too many readings, since there is a distinct risk of overtiring the eye, which produces a rapid diminution of accuracy.

In all of these tests for the purpose of determining the accuracy of the spectrophotometric method as employed by us, precisely the same conditions obtained as in the routine determinations. The results, therefore, show the accuracy really attained under our actual working conditions, and not that theoretically possible, or attainable only under especially favorable circumstances.

SELECTION OF MATERIAL

Inasmuch as the concentration of hemoglobin may vary to a considerable degree in individuals who may regard themselves as being perfectly well, the greatest care was exercised to ascertain that the individual examined was in reality normal. The numbers available at all ages were sufficiently great to make it a matter of ease to reject all unsuitable observations. In spite of the painstaking efforts to secure perfectly normal subjects, no attempt was made at any especial selection. In other words, individuals remarkable for their strength or physical development were only chosen as they happened to fall naturally within the scope of our observations. To avoid any possible error due to a preponderance of some particular type of subject, our observations were fairly evenly distributed among all walks of life, so that it is believed that the results represent a fair average. All observations were excluded if the individual had recently had any sickness, was evidently in poor nutrition, or felt below par in any way. In the case of babies and young children only such were taken as were evidently thriving, gaining normally in weight, and who had not had any sickness of conesquence within a year. By far the larger part of our babies were breast fed.

In the age groups included between the 10th and 30th years a considerable number of observations were made on schoolchildren and college students, and these observations were for the most part made shortly after the summer vacation, since the general state of health and nutrition was at this time presumably at its best. In addition to the above precautions, which are of considerable importance if the resulting figures are to establish really normal values, care was taken, as will be evident at a glance from the table, to have substantially the same number of observations in each of the various age groups, and still further to have approximately the same number for each sex. The minimum number of observations on which any figure for an age period is based is thirty, except in the comparison of the sexes, in which, of course, the number for each sex is one half the total for the group. Our results are based on a study of 919 persons, 464 male, and 455 female. With the exception of a few children, all of the determinations were made in the midafternoon hours.

METHOD OF PREPARATION OF HEMOGLOBIN

Several hundred cubic centimeters of blood were obtained from each of two healthy adults by venepuncture, and defibrinated as usual. This was then centrifugalized and the corpuscles washed several times, thus removing the major portion of the serum proteins. It was then laked by the addition of ether, added in successive small portions until a clear solution was obtained. If the solution was too viscid, sufficient water was added to bring it to the proper degree of fluidity. The blood was then treated with an approximately equal volume of aluminum cream, as suggested by Marshall and Welker.[8] The aluminum cream was prepared in the manner described by Tracy and Welker.[9] After thorough mixing and filtering, the filtrate being perfectly clear, this was placed in a refrigerator, cooled down to —3 or —4 C. and then treated with absolute alcohol until the percentage of the latter equaled 25 or 30. This was allowed to remain at the above temperature until crystallization was completed. The mass of hemoglobin crystals on the filter was then transferred to a weighing bottle of known weight, and the moist weight of the mass of crystals determined. From this a suitable sample was removed for spectrophotometric examination, placed in a very small beaker, and then dissolved in a very little 0.1 per cent. sodium carbonate solution, and transferred quantitatively to a 10 c.c. standardized flask, which was then filled up

8. Marshall and Welker: The Precipitation of Colloids by Means of Aluminum Hydroxid, Jour. Am. Chem. Soc., 1913, xxxv, 820

9. Tracy and Welker: The Use of Aluminum Hydroxid Cream for the Removal of Albumin in Nitrogen Partition in Urinary Analysis, Jour. Biol. Chem., 1915, xxii, 55.

to the mark with the same solution. A preliminary reading was made to determine the approximate concentration, and with this as a guide the solution was further diluted so as to bring the readings in the region between 60 and 80 on the scale, since this is the position of optimum accuracy. Readings were then taken in both positions in the spectrum in the way already described.

The weighing bottle with its contents was then placed in a desiccator over sulphuric acid, where it remained until it attained a constant weight. From this the dry weight of the sample was calculated, and this weight, divided by the dilution, gives the concentration, that is, the amount of hemoglobin in 1 c.c. of the solution tested. To make sure that no reduction or conversion of the oxyhemoglobin had taken place, readings were invariably taken with great care in both regions of the spectrum. The ratio of these extinction coefficients gave a constant result of 1.581, within the allowable limits of error. Blood was obtained from two separate individuals, and crystals prepared as above described from each,

DETERMINATIONS OF THE ABSORPTION RATIO (A'_0)

In the preliminary report[10] of this work all the values as there given were relative only, that is, they represented the extinction coefficients obtained by taking —log. cos.^{2} of the angles of rotation. In order to convert these relative into absolute values, that is, to determine the actual hemoglobin concentration, it is necessary to multiply the extinction coefficients by a constant factor, namely, the absorption ratio $A'o$. While the value of $A'o$ has been determined by several authors, notably Huefner, later researches made by Butterfield[2] in Huefner's laboratory give a quite different value. Moreover, Butterfield has shown that the absorption ratio may vary considerably with very slightly different conditions of experiment.

Since the accuracy of the absolute values depends on the correctness of the absorption ratio, it was felt to be important to establish this for our conditions of experiment.

In order to do this it was necessary first to prepare pure crystalline human hemoglobin. After the purity of this had been established spectrophotometrically by determining the extinction ratio, a solution of known concentration was made up. This solution was then placed in the spectrophotometer, the angle of rotation measured and from this the extinction coefficient ($E'o$) of the solution calculated. Since the concentration is known, the absorption ratio ($A'o$) can be determined from the formula $A'o = C/E'o$.

For the preparation of the hemoglobin I am indebted to the courtesy of Professor Welker, and the results are extracted from a joint

10. Williamson: Jour. Am. Med. Assn., 1915, lxv, 302.

paper in process of preparation, in which, in addition to human hemoglobin, a number of hemoglobins from different animals are being studied, Professor Welker undertaking the preparation of them, the present author determining the optical constants.

HUMAN HEMOGLOBIN

Crystallization No. 1: The dry weight of the specimen was 0.0997 gm. This was dissolved in 0.1 per cent. sodium carbonate solution, and made up to 10 c.c. in a standardized flask. From this solution three subdilutions were made, using 1 c.c. of the above for each, and diluting by adding 5.5 c.c. of sodium carbonate solution, making the total dilution 1 in 65.

Each of these subdilutions was placed in the *Trögchen* and ten readings taken in each of the two positions in the spectrum already alluded to. Table 5 gives the results.

TABLE 5.—DETERMINATION OF *A'o*

Subdilution	Average of Ten Readings (542.0 to 534.0)	Extinction Coefficient E'_0	Average of Ten Readings (566.5 to 558.5)	Extinction Coefficient E_0
No. 1	77.19	1.309	67.40	0.831
No. 2	77.36	1.320	67.04	0.818
No. 3	77.21	1.310	68.13	0.858

The average $E'o = 1.313$; and the average $Eo = 0.836$. The concentration $= 0.0997/65 = 0.001534$. $A'o = C/E'o = 0.001534/1.313 = 0.001168$. $Ao = C/Eo = 0.001534/0.836 = 0.001835$.

That the solution was actually a pure oxyhemoglobin solution is shown by the extinction ratio. $E'o/Eo = 1.313/0.836 = 1.571$.

Crystallization No. 2: The dry weight of the specimen was 0.02479 gm. This was dissolved in 0.1 per cent. sodium carbonate solution, and made up to 10 c.c. in a standardized flask. From this solution three subdilutions were made until the final dilution was 1 in 17.

TABLE 6.—DETERMINATION OF *A'o*

Subdilution	Average of Ten Readings (542.0 to 534.0)	Extinction Coefficient E'_0	Average of Ten Readings (566.5 to 558.5)	Extinction Coefficient E_0
No. 1	75.73	1.216	65.91	0.778
No. 2	76.25	1.248	66.01	0.782
No. 3	75.62	1.210	66.07	0.784

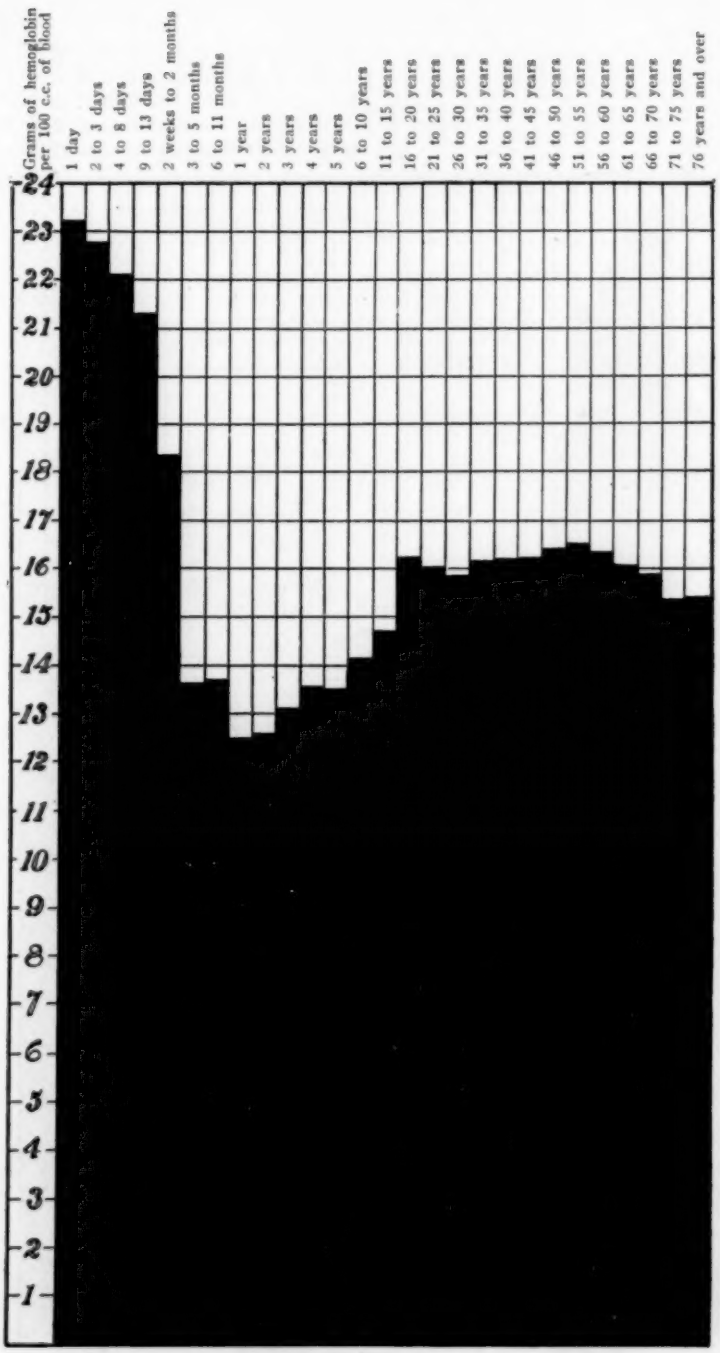

Fig. 1.—Grams hemoglobin per 100 c.c. of blood in persons ranging in age from 1 day to over 76 years.

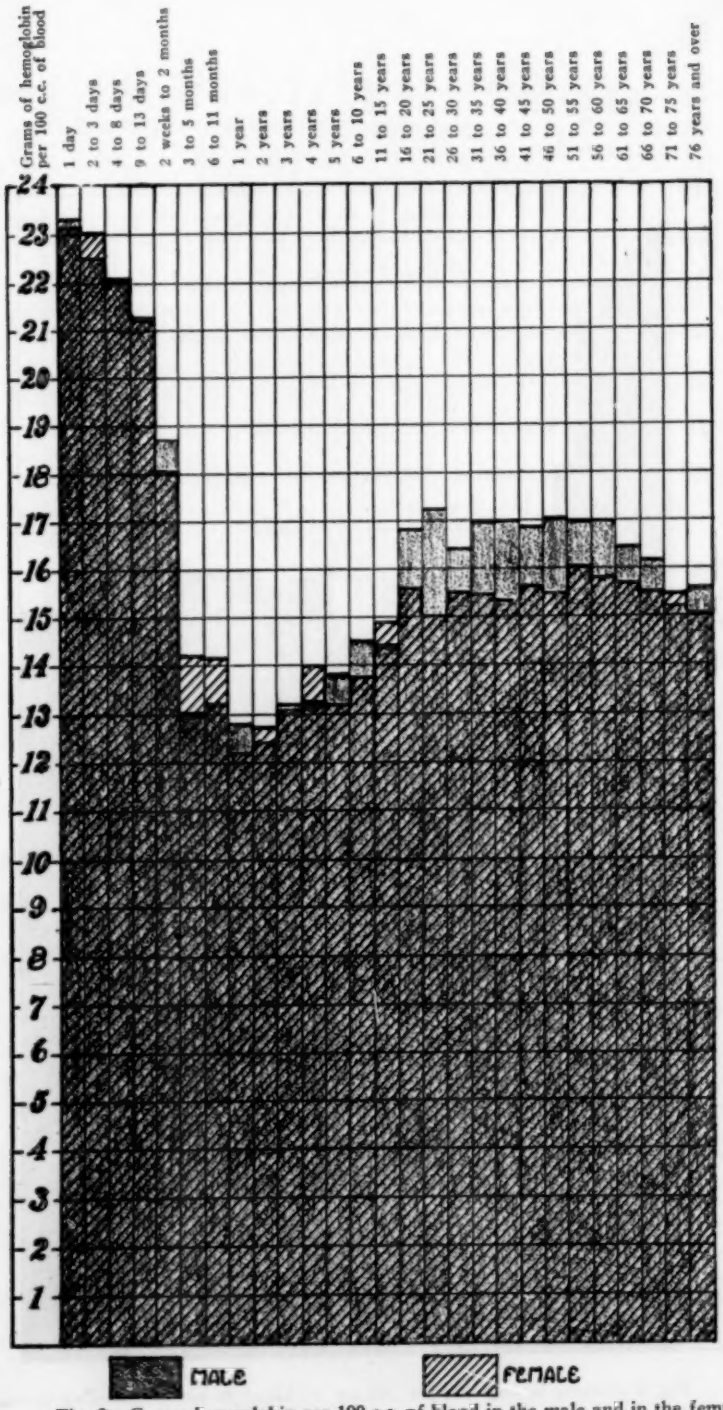

Fig. 2.—Grams hemoglobin per 100 c.c. of blood in the male and in the female, for the ages ranging from 1 day to over 76 years.

The average $E'o = 1.225$; and the average $Eo = 0.781$. The concentration $= 0.02479/17 = 0.001458$. $A'o = C/E'o = 0.001458/1.225 = 0.001190$. $Ao = C/Eo = 0.001458/0.781 = 0.001867$.

That the solution was actually a pure oxyhemoglobin solution, is shown by the extinction ratio. $E'o/Eo = 1.225/0.781 = 1.569$.

TABLE 7.—Results of Examination

Age	Extinction Coefficient, Dilution 1:110.71			Grams of Hemoglobin per 100 C.c. of Blood			Number of Cases		
	Male	Female	Both	Male	Female	Both	Male	Female	Both
1 day	1.7859	1.7763	1.7813	23.31	23.19	23.25	16	15	31
2 to 3 days	1.7239	1.7657	1.7454	22.50	23.05	22.78	15	16	31
4 to 8 days	1.6965	1.6936	1.6949	22.14	22.11	22.12	15	18	33
9 to 13 days	1.6366	1.6342	1.6355	21.36	21.33	21.35	15	15	30
2 weeks to 2 mos.	1.4329	1.3822	1.4109	18.70	18.04	18.42	15	15	30
3 to 5 months	1.0019	1.0915	1.0467	13.08	14.25	13.66	16	16	32
6 to 11 months	1.0129	1.0876	1.0499	13.22	14.19	13.70	18	15	33
1 year	0.9806	0.9367	0.9599	12.80	12.23	12.53	18	16	34
2 years	0.9530	0.9727	0.9631	12.44	12.70	12.57	16	17	33
3 years	1.0121	1.0041	1.0081	13.21	13.11	13.16	15	16	31
4 years	1.0177	1.0710	1.0435	13.28	13.98	13.62	16	15	31
5 years	1.0596	1.0166	1.0375	13.83	13.27	13.54	17	18	35
6 to 10 years	1.1163	1.0498	1.0851	14.57	13.70	14.18	18	15	33
11 to 15 years	1.1060	1.1389	1.1252	14.48	14.87	14.69	17	20	37
16 to 20 years	1.2879	1 1986	1.2447	16.81	15.64	16.25	16	15	31
21 to 25 years	1.2206	1.1515	1.2270	17.23	15.03	16.02	17	21	38
26 to 30 years	1.2572	1 1805	1.2169	16.41	15.53	15.88	19	28	47
31 to 35 years	1.2975	1.1832	1.2403	16.94	15.44	16.19	16	16	32
36 to 40 years	1.3066	1.1771	1.2428	16.98	15.36	16.22	17	15	32
41 to 45 years	1.2913	1.1961	1.2432	16.85	15.64	16.23	15	16	31
46 to 50 years	1.3080	1.1866	1.2588	17.07	15.49	16.43	22	15	37
51 to 55 years	1.2903	1.2323	1.2687	16.96	16.08	16.56	19	16	35
56 to 60 years	1.3000	1.2074	1.2537	16.97	15.76	16.36	15	15	30
61 to 65 years	1.2612	1.2033	1.2313	16.46	15.71	16.07	15	16	31
66 to 70 years	1.2405	1.1880	1.2159	16.19	15.51	15.87	17	15	32
71 to 75 years	1.1663	1.1848	1.1750	15.22	15.46	15.34	19	17	36
76 and over	1.2003	1.1519	1.1793	15.67	15.04	15.39	30	23	53
							464	455	919

Average for the two crystallizations: No. 1 $A'o = 0.001168$; No. 2 $A'o = 0.001190$; average $A'o = 0.001179$. No. 1 $Ao = 0.001835$; No. 2 $Ao = 0.001867$; average $Ao = 0.001851$.

In common with other observers, we find that the readings in the band ($A'o$) are slightly more accurate than those taken between the bands. In accordance with this all our absolute figures have been calculated by multiplying the extinction coefficient of the readings in this position, $E'o$, by the average absorption ratio, $A'o = 0.001179$. This result, multiplied by the dilution 110.71, gives the concentration, that is, the amount of hemoglobin in each cubic centimeter of blood. For convenience sake we have expressed the results in grams of hemoglobin per 100 c.c. of blood, since this permits us to avoid fractions.

CHARACTERISTICS OF THE AGE CURVE

By far the highest values attained are at birth ($E'o = 1.781 = 23.25$ gm.). This substantiates the results of many investigators. Beginning immediately, there is a very rapid decline, so that from the 3rd to the 5th month the average value is nearly down to the minimum ($E'o = 1.047 = 13.66$ gm.) and is far below the average adult figure. From the 5th month onward there is a relatively gradual diminution, but the actual minimum ($E'o = 0.960 = 12.53$ gm.) is not reached until 1 year, that is in the 2d year of life. During the next year also the same minimum value is found, and only after the child has completed its 2d year does the curve rise again. This is then fairly rapid up to the 16th year, and from the 16th to the 55th year the variations in the different age periods are very slight.

The immediate and rapid decline in the amount of hemoglobin after birth and continuing up to the 6th month, and to a less extent up to the 1st year, is a highly characteristic feature of the age curve. Our results are in this respect in accord with those of Leichtenstern. This decline is quite independent of nutrition, since, as has been above stated, the greatest care was exercised to select only infants that were thriving. Moreover, it is not a question of artificial feeding, since the great majority of our cases, especially up to the 6th month, were breast-fed infants.

A noticeable feature of our curve is the fact that in the age period from 16 to 20 years the values have attained practically the maximum ($E'o = 1.245 = 16.25$ gm.) and from this period to middle age the curve is nearly horizontal. The very highest point is attained in the period from 51 to 55, but the variation in this period from the other younger adult values is insignificant. From the 55th year onward the values decline steadily up to the 75th year ($E'o = 1.175 = 15.34$ gm.). After the 76th year there is a rise, but it is so small as to be negligible ($E'o = 1.179 = 15.39$ gm.). The sharp drop found by Leichtenstern in the period from 55 to 60 is seen, on the basis of our larger number of patients, not to exist. The rise in old age found by him, was based on only five cases in all, whereas our figures for the age period over 76 alone is based on a total of fifty-three persons.

INFLUENCE OF SEX

An inspection of Figure 2 shows that the values from birth up to the 15th year are almost identical for both sexes. At birth the difference is so slight that it can just be portrayed graphically on our curve: male $E'o = 1.786 = 23.31$ gm.; female $E'o = 1.776 = 23.19$ gm.

It must be remembered that the figures on which the values for each sex are based, represent, of course, only one-half the total number of cases. It is, therefore, to be expected that slight variations will occur in these curves to a somewhat larger extent than when the figures for both sexes are combined in a common curve. One especially valuable observation was made on twins, a boy and a girl, both perfectly healthy, of approximately the same weight, whose blood was first examined when they were 5 hours old. The values were almost identical. In order to formulate more precisely the relative values for the two sexes up to the age of puberty we have calculated the average extinction coefficient for each sex separately, as shown by Table 8.

TABLE 8.—INFLUENCE OF SEX ON HEMOGLOBIN IN CHILDHOOD

Age	Sum of Extinction Coefficients, Male	Number Cases	Sum of Extinction Coefficients, Female	Number Cases
1 day	28.576	16	26.645	15
2 to 3 days	25.858	15	28.232	16
4 to 8 days	25.447	15	30.486	18
9 to 13 days	24.550	15	24.513	15
2 weeks to 2 months	21.503	15	20.733	15
3 to 5 months	16.031	16	17.464	16
6 to 11 months	18.333	18	16.314	15
1 year	17.650	18	14.988	16
2 years	15.248	16	16.536	17
3 years	15.151	15	16.071	16
4 years	16.284	16	16.005	15
5 years	18.014	17	18.299	18
6 to 10 years	20.603	18	15.747	15
11 to 15 years	18.853	17	22.779	20
	281,711	227	284.802	227

The average extinction coefficient for males is $1.241 = 16.20$ gm.; for females, $1.255 = 16.38$ gm.

From these averages it is clear that the variations in hemoglobin due to sex are, up to the 16th year, hardly greater than the error in the method, and may, for all practical purposes, be neglected.

On the other hand, from the 16th to the 70th year the sex differences make themselves plainly manifest. While the variations are, for either sex, relatively slight within these periods, the values for women are, in every instance, considerably lower than those for men. From the 50th to the 70th year, that is, after the cessation of menstruation, it should be noted that the differences between the sexes still exists, although to a less marked degree than during the child-bearing period.

Table 9 shows the differences due to sex from the 16th to 70th year.

TABLE 9.—Influence of Sex on Hemoglobin in Adult Life

Age	Sum of Extinction Coefficients, Male	Number Cases	Sum of Extinction Coefficients, Female	Number Cases
16 to 20 years	20.606	16	17.979	15
21 to 25 years	22.445	17	24.181	21
26 to 30 years	23.867	19	33.308	28
31 to 35 years	20.759	16	18.932	16
36 to 40 years	22.114	17	17.637	15
41 to 45 years	19.370	15	19.109	16
46 to 50 years	28.778	22	17.800	15
51 to 55 years	24.688	19	19.718	16
56 to 60 years	19.500	15	18.116	15
	202.147	156	186.860	157

The average extinction coefficient for males is 1.296 = 16.92 gm.; for females, 1.190 = 15.53 gm.

It will be seen from Table 9 that from the ages of 16 to 60 inclusive the hemoglobin of the two sexes are in the ratio of 1.296 to 1.190; or, otherwise expressed, the female averages 91.8 of the value of the male. Expressed in absolute terms, the men average 16.92 gm., the women 15.53 gm.

TABLE 10.—Influence of Sex on Hemoglobin for the Ages, Sixty-One to Seventy Years

Age	Sum of Extinction Coefficients, Male	Number Cases	Sum of Extinction Coefficients, Female	Number Cases
61 to 65 years	18.919	15	19.254	16
66 to 70 years	21.089	17	17.821	15
	40.008	32	37.075	31

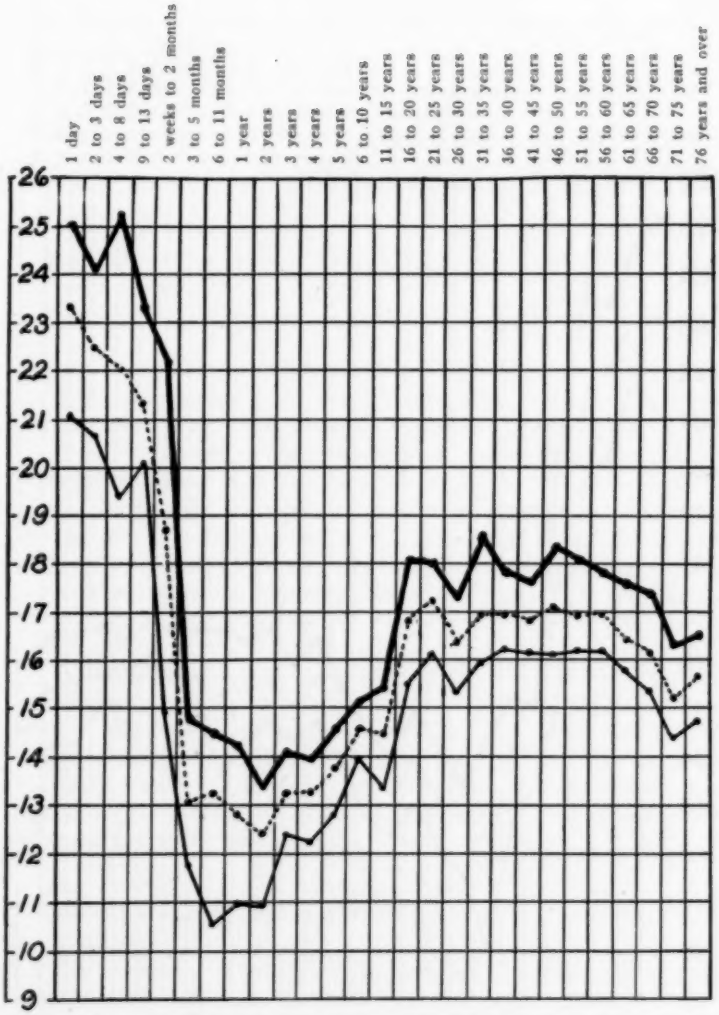

Fig. 3.—The average amount of hemoglobin, and the average amount for those having more than the average and also for those having less than the average, for males ranging in age from 1 day to over 76 years.

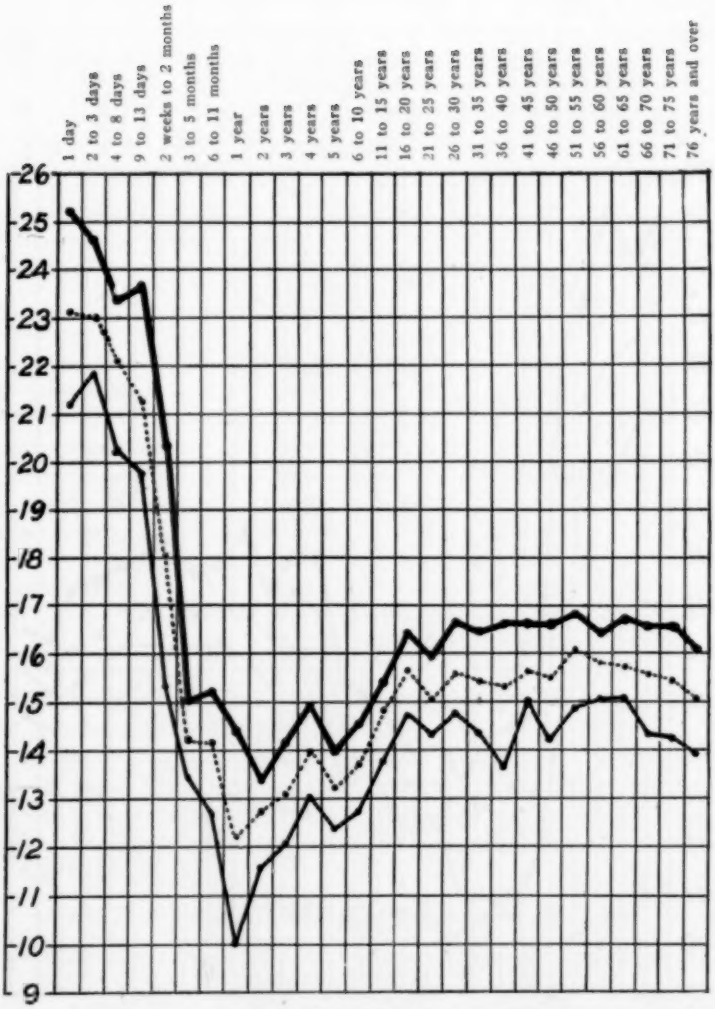

Fig. 4.—The average amount of hemoglobin, the average amount for those having more than the average and also for those having less than the average, for females varying in age from 1 day to over 76 years.

For the ages from 61 to 70 inclusive the values are as 1.250 to 1.196, that is, the value for the women is 95.6 per cent. of the value for the men. Expressed in absolute terms, the men average 16.32 gm., the women 15.61 gm.

TABLE 11.—Influence of Sex on Hemoglobin for the Ages Above Seventy-One Years

Age	Sum of Extinction Coefficients, Male	Number Cases	Sum of Extinction Coefficients, Female	Number Cases
71 to 75 years.....	22.161	19	20.142	17
Over 76.....	36.010	30	26.495	23
	58.171	49	46.637	40

The averages are 1.187 for the men, and 1.166 for the women, or, expressed in absolute terms, 15.49 and 15.22 respectively.

It is, of course, quite desirable to know within what limits the hemoglobin values vary in individuals of the same age and sex. To obtain what might be properly regarded as an average variation we adopted the following method: We first averaged all of the values in a given group, then took all the values above this average, and averaged them separately, repeating the process with all the values below the average. This was done for male and female separately, and the results are shown in Figures 3 and 4.

An inspection of these figures shows that the variations from the average are somewhat greater in very early life. This is, of course, almost self-evident, when we realize how marked a difference in hemoglobin may be produced by a very slight difference in age during the early months of life. From the 3rd or 4th year onward this average variation is fairly constant, and amounts to approximately 1 gm. above or below the average normal.

CONCLUSIONS

From a simple inspection of the Figures the following conclusions are obvious:

1. The amount of hemoglobin in the blood of normal persons varies greatly at different ages, and follows a well-defined curve.

2. These age variations are so great that in determining whether a given blood contains more or less hemoglobin than normal, it is imperative to consider the age. These variations are greatest from birth to the 16th year.

3. Between the ages of 16 and 60 there is a marked difference between the two sexes, this difference growing less after the 60th year.

4. In view of these facts, it is evident that hemoglobinometers should be standardized in absolute terms, most conveniently in grams of hemoglobin per 100 c.c. of blood. (Because of the superior accuracy attained, it is highly desirable that the standardization of hemoglobinometers should be spectrophotometrically controlled.)

5. Whether or not a given blood contains a greater or less amount of hemoglobin than the normal can be determined only by a comparison of the absolute value obtained by a hemoglobinometer thus standardized, with the normal value for that age and sex, as shown by Table 7.

THE AUTOLYSIS OF NITROGENOUS COMPOUNDS OF THE BLOOD SERUM IN GENERAL PARALYSIS AND DEMENTIA PRAECOX

WITH ITS BEARING ON THE ABDERHALDEN TEST *

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In previous communications[1] from this laboratory it was demonstrated that the blood serum from normal animals, treated according to the Abderhalden method, with substrates prepared from various tissues, gave positive reactions, thus proving that disease of the corresponding organs is not necessary for the occurrence of a positive Abderhalden reaction with such substrates. It was further shown that both with animal and human serums the appearance of a positive reaction varied with the thoroughness with which the substrates were washed with boiling water. All substrates used could, by repeated washing, be rendered incapable of producing positive Abderhalden tests. It was further established that the serum of persons suffering from general paralysis of the insane, when tested with brain substrate, showed a quantitatively different reaction from that of normal individuals. This difference, calculated by personal estimates of the degree of ninhydrin reaction, was illustrated by a curve which is here reproduced in Figure 1.

In order to establish this fact by quantitative methods we have performed a series of tests with brain substrates and the blood serums of normal persons, of patients presenting unmistakable evidence of general paralysis of the insane and others showing a dementia praecox type of reaction.

Estimations of the amount of amino-acid nitrogen were made, by the Van Slyke method, in the serum immediately after separation and again at the end of twenty-four hours, during which the samples were kept in a shaking machine at a temperature of 37 C. These samples included serum alone and serum mixed with 0.25 gm. of brain substrate. At the end of the period the serum was separated from the substrate by filtration. In Table 1 are given the figures thus obtained and the average results are shown in graphic form in Figure 2 for convenience of comparison with the results shown in Figure 1.

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* Submitted for publication July 3, 1916.

* From the Laboratory of the Illinois State Psychopathic Institute.

* Read before the American Neurological Society, May 10, 1916.

1. Ross and Singer: THE ARCHIVES INT. MED., 1914, xiv, 552; Ibid., 1915, xv, 724.

These results seem to suggest very strongly that the coagulable nitrogen of the substrate can have nothing to do with the determination of a positive Abderhalden test, a conclusion which is strongly supported by the fact, previously published, that the materials removed from various organs by washing with slightly acidulated boiling water in the process of preparing the organs for substrates will themselves serve as efficient substrates for the production of a positive Abderhalden reaction.

TABLE 1.—Increase of Amino-Acid Nitrogen in Milligrams per Cubic Centimeter of Blood Serum When Incubated for Twenty-Four Hours at 37 C.

	Donor of Serum	Before Incubation	Increase After 24 Hours Without Substrate	Increase After 24 Hours with Brain Substrate Which Had Been Washed*			
				2 Times	4 Times	6 Times	11 Times
	1	0.886	0.023	0.006	0.057	0.012	0.021
	2	0.604	0.009	0.111	.....	0.038	0.020
Normal	Average	0.745	0.016	0.103	0.057	0.025	0.021
General paralysis of the insane	E. B.	0.520	0.001	0.168	.....	0.028	0.028
	A. C.	0.796	0.008	0.073	.....	0.059	0.012
	A. S.	0.732	0.018	0.116	.....	0.046	0.001
	G. M.	0.597	0.007	0.220	.....	0.188	0.009
	J. L.	0.399	0.026	0.192	0.122	0.110	0.085
Average		0.609	0.012	0.154	0.122	0.087	0.045
Dementia praecox	S. B.	0.004	0.019	0.186	.....	0.060	0.041
	C. D.	0.684	0.050	0.154	.....	0.028	0.028
	J. S.	0.815	0.014	0.074	0.052	0.030	0.000
	A. B.	0.794	0.010	0.074	0.037	0.004	0.000
	F. W.	0.596	0.019	0.128	0.056	0.040	0.045
Average		0.609	0.016	0.123	0.048	0.053	0.023

* The water which had been used for the second and subsequent washings gave no reaction with ninhydrin.

Investigations were next undertaken of the antitryptic titer of serum, during which it was noted that the addition of placenta substrate to serum resulted in a considerable increase in the total nitrogen of the serum. It was found that this increase reached its maximum in about ten minutes. The substrate used was placenta which had been washed twice. The wash water was negative to ninhydrin and a negative result was obtained in the dialysate when this substrate was incubated with water or normal salt solution according to the Abderhalden technic.

To a number of tubes containing 0.25 gm. of this placenta were added 1 c.c. of blood serum. Of these tubes, one was immediately filtered after dilution to 20 c.c., a second was filtered at the end of ten minutes and the others after thirty, and 180 minutes respectively, the same dilution being made in each case. Kjeldahl determinations

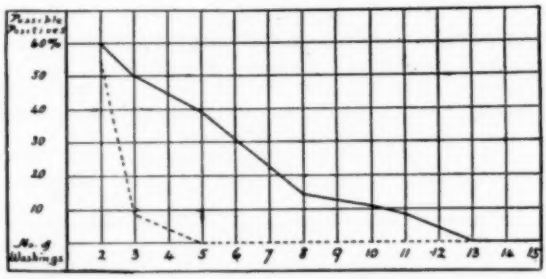

Fig. 1.—Proteolysis of brain substrate at different stages of washing by serum from normal persons and from patients with general paralysis. The solid line represents the results in general paralysis; the broken line, normal serum.

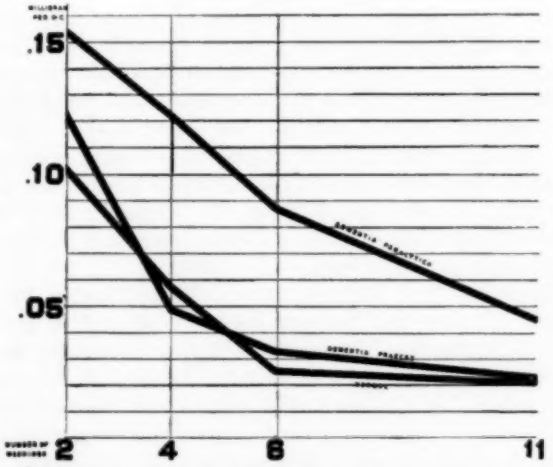

Fig. 2.—Increase of amino-acid nitrogen in serums incubated with brain substrate in various stages of washing.

were made on the blood serum alone, 0.25 gm. placenta alone, the filtrates from each of the above tubes and the residue with the filter paper in each instance. From these data it was possible to determine the increase in the total nitrogen of 1 c.c. of serum.

It was therefore decided to perform a series of tests on different blood serums and to compare the total autolysis of serum which had been treated for ten minutes with placenta substrate and then filtered with that which occurred in serum not so treated. The time allowed for the digestion was in all cases twenty-four hours and the method of determination used was that for noncoagulable nitrogen of Folin and Denis.[2] The total nitrogen of the serum was also determined by the Kjeldahl method. The results with normal, general paralytic and dementia praecox serums are given in Table 3.

TABLE 2.—Increase of Total Nitrogen of Serum from the Addition of Placenta Substrate

	Nitrogen, Mg.	Increase, Mg.
Original serum	11.653	
After momentary mixture with placenta	13.104	1.451
After 10 minute mixture with placenta	14.040	2.387
After 30 minute mixture with placenta	14.132	2.479
After 180 minute mixture with placenta	14.040	2.387

Figure 3 is a graphic representation of the average results for the three groups of blood serums given in Tables 1 and 3, those from the former being taken from the column for brain substrate which had been washed twice. The solid black represents the results from incubation of the serum alone, while the full height of the columns corresponds with those in serum which had been treated with substrate. It should be remembered in considering this chart that the first three columns refer to amino-acid nitrogen, the second three to total noncoagulable nitrogen and that the substrates used in the two sets were brain and placenta respectively.

In discussing these findings, one of the first points for especial emphasis is the fact that placental substrate allowed to act for only ten minutes is sufficient to remove antitryptic substances to such an extent that the increase in noncoagulable nitrogen during incubation for twenty-four hours exceeds that which occurs without the use of the substrate in serum from normal persons and from dementia praecox patients by approximately 260 per cent., and in general paralysis by 1,260 per cent. Furthermore, from the conditions of the experiment it is obvious that the nitrogen compounds which are split up must be those of the blood serum itself. The nitrogen derived from the substrate has, in each instance, been deducted from the figures here given for total noncoagulable nitrogen.

2. Jour. Biol Chem., 1911-1912, xi, 527.

The further observations, alluded to above, that the water which has been used for washing substrates contains materials capable of giving rise to positive Abderhalden tests and that substrates can be washed so thoroughly that they are no longer efficient, make it extremely probable that the active agent in removing antitryptic bodies from the serum is some soluble substance or substances. Necessarily,

TABLE 3.—Increase of Total Noncoagulable Nitrogen in Milligrams per Cubic Centimeter of Blood Serum When Incubated for Twenty-Four Hours Without, and After, Treatment for Ten Minutes with Placenta Substrate Which Had Been Washed Twice, the Wash Water Being Negative to Ninhydrin

	Donor of Serum	Total Nitrogen in Serum	Noncoagulable Nitrogen		
			Before Incubation	Increase after Twenty-Four Hours without Substrate	Increase after Twenty-Four Hours Subsequent to Treatment with Placenta for Ten Minutes Only
Normal	1	13.199	1.102	0.183	0.295
	2	14.793	0.938	0.069	0.332
	3	13.642	0.706	0.119	0.374
	4	13.465	0.903	0.154	0.323
Average	...	13.775	0.927	0.131	0.335
General paralysis of the insane	A. S.	11.288	1.346	0.000	0.197
	N. P.	12.667	1.047	0.071	0.392
	H. S.	13.376	1.065	0.003	0.448
	C. W.	13.908	0.964	0.011	0.265
	E. M.	14.137	1.033	0.045	0.340
Average	...	13.075	1.091	0.026	0.328
Dementia praecox	A. C.	15.275	1.152	0.032	0.076
	G. C.	14.174	0.975	0.244	0.634
	T. H.	14.085	0.808	0.064	0.292
	R. B.	14.571	0.970	0.174	0.325
Average	...	14.551	0.970	0.128	0.322

the concentration of these materials present in the wash water will vary according to the amount of water used in relation to the bulk of tissue being washed, the thoroughness of previous washings and the amount of these substances present in the tissue. The efficiency of the wash water will thus vary and we have found that it may be increased by careful evaporation. Some preliminary observations have been made with the wash water from placental tissue which seem sufficiently

significant to warrant mention here, although they are not by any means complete or exhaustive.

In one sample of such wash water it was found by the Kjeldahl method that nitrogen was present to the amount of 34.515 mg. per 100 c.c. Both urea and ammonium salts were present and determinations made of these two bodies gave 25.13 mg. urea nitrogen and 13.60 mg. ammonia nitrogen per 100 c.c., making a total of 38.73 mg. per 100 c.c., slightly larger than the Kjeldahl estimate for total nitrogen. It therefore seemed probable that the nitrogen present existed very largely in these two forms.

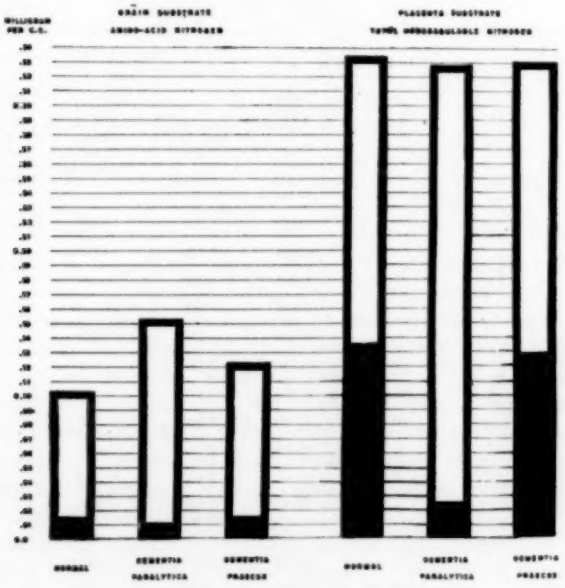

Fig. 3.—Effect of the removal of antitryptic substances from blood serums on the increase during incubation of amino-acid and of total noncoagulable nitrogen. Increases in the untreated serum are indicated in solid black, while those in the treated serum are represented by the full height of the columns.

Dialysis[3] tests have been performed with blood serum mixed with placenta substrate, a solution of urea (1 c.c. contained 2.158 mg. of nitrogen), a solution of ammonium chlorid (1 c.c. contained 0.990 mg. of nitrogen) and a mixture of these last two. The tubes were

3. The dialyzing thimbles used were made of celloidin and we are not yet satisfied that they are free from objection.

incubated for sixteen hours and 10 c.c. of the dialysate tested with ninhydrin. The following results were obtained:

Serum alone	Negative
Serum with placenta	Positive
Serum with urea	Negative
Serum with ammonium chlorid	Positive
Serum with urea and ammonium chlorid	Positive

The positive reaction in each instance was faint and about equally so in all three. The serum used was that of a male general paralytic. It should be stated that in another test a positive was obtained with urea.

Attention may be called to certain other features in the tables and charts. The close similarity between dementia praecox and normal serum in all tests, with the wide divergence of that from general paralysis, is striking. Of course it is not possible to state without actual test that this is true for all substrates, but this seems at least probable if we are correct in regarding the substrate merely as a means of removing antitryptic properties.

It may be questioned, on the same grounds, whether we are justified in comparing the results of tests with placenta with those obtained when brain substrate is used. But it may be pointed out that the figures given offer a possible explanation for the fact that positive Abderhalden reactions are obtained with some serums and not with others. It will be observed that the ratio of the amino-acid nitrogen in the first set of tests to that of total noncoagulable nitrogen in the second set considered as 100 is as follows:

	Normal	General Paralysis	Dementia Praecox
Without substrate	13.0	46.0	12.5
With substrate	30.8	47.0	37.0

Amino-acid nitrogen gives a positive ninhydrin reaction; the lower products of lysis probably do not under the ordinary conditions of the Abderhalden test. Thus the blood serum of paretics not only has a higher antitryptic value, but, when this is neutralized or partly neutralized, the process of proteolysis takes place either less rapidly or less completely. The serum from dementia praecox also shows a somewhat greater proportion of amino-acid nitrogen and would therefore tend to develop a slightly larger percentage of positive Abderhalden reactions than the normal.

A comparison between the antitryptic titer of the different groups of serum, as measured by the two sets of tests, yields very strikingly similar results. The proportion of amino-acid nitrogen increase without substrate treatment to that with is 15.5 per cent. for the normal, 7.8 per cent. for general paralysis and 13 per cent. for dementia praecox. Corresponding figures for total noncoagulable nitrogen are 37.1

per cent., 20.3 per cent., and 38.5 per cent., respectively. If these are expressed in the form of ratios with general paralysis as 10, the results are as follows:

	Normal	General Paralysis	Dementia Praecox
Amino-acid nitrogen	20	10	17
Total noncoagulable nitrogen..	18	10	19

The number of cases examined is small and therefore broad conclusions are not advisable, but attention may be directed, in a further comparison of these three groups of serums, to the relation of noncoagulable nitrogen to total nitrogen. For both normals and dementia praecox it is 6.7 to 100 (although the figures are higher for dementia praecox), whereas in paresis this ratio is 8.4 to 100. Comparing the figures in Table 3 with those in Table 1, the percentage of noncoagulable nitrogen represented by amino-acid nitrogen, is as follows:

	Per Cent.
Normal	79.7
Dementia praecox	72
General paralysis	56

SUMMARY

1. The efficiency of brain substrate in destroying antitryptic bodies in blood serum, as estimated by determination of the amino-acid nitrogen produced, is gradually removed by repeated washing with boiling water.

2. Substrates prepared from placenta contain water-soluble substances capable of neutralizing antitryptic bodies. A sufficient amount of these substances can be extracted by the serum during ten minutes contact to permit free autolysis.

3. In the cases examined the average antitryptic titer of the blood serum was high for general paralysis and approximated the normal for dementia praecox.

4. After neutralization of antitryptic bodies the increase of noncoagulable nitrogen was approximately the same for normals, general paralytics and dementia praecox patients, but the proportion of amino-acid nitrogen was much greater in general paralysis and slightly greater in dementia praecox than in the normal.

5. The second wash water from placenta substrate contained urea and ammonium nitrogen, but was negative to ninhydrin. Positive Abderhalden tests have been obtained with ammonium chlorid as a substrate.

6. The noncoagulable nitrogen of the serum is increased by ten minutes' contact with placenta substrate.

CONCLUSIONS

Substrates neutralize antitryptic bodies in the blood serum, thus permitting fermentation of the nitrogenous constituents of the serum. This autolysis will, if not carried too far, cause a positive Abderhalden reaction.

The Abderhalden test is of no value as an index of disease or disorder of function in any particular organ.

AMMONIUM SALTS AND THE ABDERHALDEN TEST*

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HOSPITAL, ILL.

In an earlier communication[1] we have shown that placental substrate, when extracted with water or normal salt solution, yielded considerable quantities of ammonia nitrogen, and that positive Abderhalden (ninhydrin) reactions had been obtained with blood serum dialyzed with ammonium chlorid as a substrate.

It has also been demonstrated[2] that, contrary to the opinion expressed by Abderhalden and others, all ammonium salts, if sufficiently concentrated, react positively to ninhydrin. None, with the exception of ammonium-sodium-hydrogen phosphate, gave a positive reaction in a dilution as great as 0.05 mg. nitrogen per cubic centimeter, and the result here was so faint as to be negligible. It was, however, found that the addition of a reducing agent, pyridin, yielded positive reactions, even in such dilute solutions, with all ammonium salts. The method of conducting the ninhydrin test in these experiments differed from that of the Abderhalden technic in that 1 c.c. of 1 per cent. ninhydrin was used and the boiling was continued for twenty minutes.

TABLE 1.—NINHYDRIN TESTS WITH AMMONIUM PHOSPHATE AND GLUCOSE MIXTURE

Ammonia Nitrogen Content, Mg.	Glucose, Mg.	Result
0.28	2	Negative
0.35	2	+ (faint)
0.49	2	+
0.63	2	++
0.35	1	Negative
0.56	1	+ (faint)
0.70	1	+

In view of the fact that blood serum normally contains at least one reducing agent, glucose, in considerable quantity, it seemed worth while to perform tests with ammonium salts and glucose and to employ the technic of Abderhalden, wherein 0.2 c.c. of 1 per cent. ninhydrin is added to 10 c.c. and the mixture is boiled for one minute only.

* Submitted for publication July 25, 1916.
* From the Laboratory of the Illinois State Psychopathic Institute.

1. THE ARCHIVES INT. MED., p. 529.
2. Harding and Warneford: Jour. Biol. Chem., 1915, xxv, 319.

Qualitative tests of the normal salt extract from placenta substrate demonstrated the presence of considerable amounts of phosphoric acid and, since ammonium phosphate seemed to react especially readily with ninhydrin, it was decided to employ the neutral ammonium phosphate for this purpose. As a reducing agent we have used glucose. Table 1 gives the results of a series of tests in each of which the volume of ammonium phosphate-glucose solution was made up to 10 c.c.

Placental substrate, which had been washed twice (the wash water was negative to ninhydrin), was extracted with normal salt solution for sixteen hours. The ammonia nitrogen in the extract was estimated as 0.62 mg. for each gram of placenta. Table 2 gives the results of boiling portions of the extract, made up in each case to 10 c.c. by the addition of water, with and without glucose, for 1 minute with 0.2 c.c. of 1 per cent. ninhydrin:

TABLE 2.—NINHYDRIN TESTS WITH NORMAL SALT EXTRACT OF PLACENTA SUBSTRATE AND GLUCOSE MIXTURE

Ammonia Nitrogen Content, Mg.	Equivalent Placenta Substrate, Gm.	Glucose, Mg.	Result
0.99	1.6	...	Negative
0.62	1	1	Doubtful
0.73	1.2	1	+

In the Abderhalden tests, as usually carried out, the substrate is washed four or five times before use and the amount of ammonia nitrogen which would be extracted in sixteen hours would be considerably less than with the substrate here employed, which had been washed with boiling water only twice. The weight of substrate used is generally from 0.25 to 0.5 gm. Furthermore, glucose is present in normal blood serum to the extent of about 1 mg. per cubic centimeter. From 1 to 1.5 c.c. are used for a test and the serum dialyzed into 20 c.c. of water, 10 c.c. of which are used for the actual ninhydrin reaction. This 10 c.c. can therefore rarely contain more than 0.5 mg. glucose.

In the results given above it was necessary to use the extract from 1.2 gm. placenta substrate with 1 mg. glucose in order to obtain a positive ninhydrin reaction following the Abderhalden technic, and there would therefore appear to be but little danger of error from this source, provided care is used in washing the substrate and too large quantities of serum and substrate are avoided.

In order to confirm this opinion we have carried out some dialysis tests with placenta substrate and a solution of glucose and others with blood serum and ammonium phosphate solution. In the former 0.5, 1, and 1.5 gm. placenta were placed in dialyzing thimbles with, in each instance, 1 c.c. of glucose solution containing 2 mg. glucose. There

were placed 20 c.c. distilled water in the tube outside the thimble and 10 c.c. of this was used after sixteen hours' incubation for the ninhydrin test. All gave a negative result.

In the second series blood serum from a normal person was used, some being drawn before breakfast and a second sample one hour after breakfast. The glucose content was determined by the method of Strouse, Stein and Wiseley[3] to be 0.725 mg. per cubic centimeter before breakfast, and 1.189 mg. per cubic centimeter after breakfast. The results of dialysis tests were as given in Table 3.

TABLE 3.—NINHYDRIN TESTS WITH THE DIALYSATE FROM AMMONIUM PHOSPHATE AND BLOOD SERUM MIXTURE

	Serum, C.c.	Ammonium Phosphate in Mg. of Ammonia N	Result
Before breakfast	1.5	0.315	+ (very faint)
	1.5	0.49	+
	1.5	0.7	+
After breakfast	1.5	0.315	Lost
	1.5	0.49	+ (very faint)
	1.5	0.7	+ (slight)

The reaction with 0.7 mg. ammonium phosphate and serum taken before breakfast was very marked, more so than that with the same amount and after-breakfast serum. Since the latter contained a larger quantity of glucose, it seems probable that the positive reactions here depend on autolysis of serum and are not merely reactions between the ammonium salt and ninhydrin in the presence of a reducing agent, the glucose of the serum. This reaction is similar to that previously reported with ammonium chlorid as a substrate.

SUMMARY AND CONCLUSIONS

Ammonium phosphate will serve as an efficient substrate in the performance of the Abderhalden test.

Ammonium phosphate in dilute solution will give a positive reaction with ninhydrin, used according to the Abderhalden technic, in the presence of glucose.

Although ammonia nitrogen and phosphoric acid are present in the watery extract from placental substrate and glucose is present in the blood serum, the quantities are too small to give rise to error when the Abderhalden technic is followed.

3. Strouse, Stein and Wiseley: Bull. Johns Hopkins Hosp., 1915, xxvi, 211.

HEMATOCHYLURIA

OBSERVATIONS ON THE FAT CONTENT OF THE URINE AND THE PATHOLOGY OF THE CONDITION *

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The following case on the medical service of Dr. Henry A. Christian at the Peter Bent Brigham Hospital gave an opportunity for the study of hematochyluria with especial reference to the fat content of the urine.

S. G. (P. B. B. H. Med. No. 1636), a man, aged 27 years, single, West Indian mulatto, a seaman, entered the Peter Bent Brigham Hospital, Sept. 14, 1914, complaining of disturbance of micturition and peculiarly colored urine. The family history was negative. The patient was born in Barbados, where he lived until 15 years of age. He had always been in good health. No history of urinary disturbances prior to the present illness was obtained. In 1903, two years after leaving Barbados, he noticed slight enlargement of the left testicle. Five years before entrance he had gonorrhea, which was not followed by any complications.

Three years previous to admission, while urinating, the flow suddenly stopped and the patient had to be catheterized. This attack was followed by pain and tenderness in the region of the symphysis pubis. He noticed at that time that the urine was very dark. One year previous to admission the left testicle became markedly swollen, but was not painful or tender. During the last nine months previous to admission there were four attacks of acute retention similar to the one described. During one of these attacks, one week previous to admission, he noticed for the first time that the urine was almost milk white with a reddish tinge. The urine has remained of this color. Aside from the attacks, he felt quite well, except for weakness. He lost 20 pounds in weight in the six months previous to entrance.

Physical examination showed a well-developed, but rather poorly nourished, young mulatto lying quietly in bed in no evident distress. The pupils were regular, equal and reacted normally to light and accommodation. There was some pyorrhea alveolaris, and the upper and lower central incisors were carious. The throat was negative. There was no enlargement of the lymph nodes, except at the angle of the right jaw. The chest and lungs were negative. Both heart sounds were heard and were normal; there was reduplication of the second sound at the base. The pulse was of good quality, regular and normal. The systolic blood pressure was 120 mm. of mercury, the diastolic 70 mm. The abdomen was negative; the spleen was not felt; the liver was not enlarged; there were no masses in the abdomen and no tenderness. In the right groin there was a scar about 3 cm. in length, the result of trauma. The left half of the scrotum was somewhat enlarged and felt like a varicocele. The left testicle was somewhat enlarged, but was not tender. The right testicle was normal. The superficial and deep reflexes were present and normal. Rectal examination was negative.

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* Submitted for publication July 3, 1916.
* From the Medical Clinic of the Peter Bent Brigham Hospital.
* This study was done under a grant from the Proctor Fund of the Medical School of Harvard University for the Study of Chronic Diseases.

On admission the urine was reddish brown and turbid. The odor was distinctly less urinous than normally. After sedimentation the supernatant fluid was distinctly milky. It was acid, with a specific gravity of 1.020, albumin 0.2 per cent. and no sugar. The sediment showed an occasional leukocyte, many red blood cells, and occasional hyaline and coarsely granular casts. Under the oil immersion lens were seen myriads of minute, highly refractile droplets, which stained with Sudan III. The sediment of many of the specimens contained numerous small blood clots. Repeated examination of these failed to reveal any filarial embryos. On standing, many of the specimens coagulated in about fifteen minutes into firm, gelatinous masses. These dissolved in about two and one-half hours. There then formed a narrow, bright red zone consisting of red blood cells, above this a broad, pinkish white zone and on top a narrow, milky zone.

Blood examination revealed red blood cells 3,160,000, white blood cells 7,200, hemoglobin (Sahli) 77 per cent.; the differential count showed polymorphonuclear neutrophils 53 per cent., eosinophils 25 per cent., basophils none, large mononuclear cells 10 per cent., small mononuclear cells 12 per cent. The erythrocytes showed a moderate achromia. Repeated examinations of the blood at night and in the day failed to show any embryos of filaria. Ten c.c. of blood examined for parasites by the method of Staubli yielded negative findings.

The stools showed numerous rhabditiform embryos of *Strongyloides stercoralis*, otherwise they were not abnormal. The Wassermann reaction on the blood serum was positive. The von Pirquet cutaneous test with tuberculin was negative. Analysis of the gastric contents, bismuth Roentgen-ray examination of the gastro-intestinal tract and electrocardiographic studies of the heart yielded normal findings. The temperature was irregularly remittent, reaching 103 on one occasion, and falling to normal before discharge.

A cystoscopic examination, made by Dr. Goetsch, showed the bladder mucosa definitely reddened with a considerable amount of surface desquamation. The ureteral orifices were of normal appearance. From the left orifice were seen frequent spurts of turbid urine, which momentarily obscured the view in that locality. From the right orifice no such turbid urine was seen. The ureters were then catheterized; there was a good flow of almost clear urine from the right side; from the left side the flow was about half as rapid as from the right and the urine was opalescent, definitely turbid and blood tinged.

The urine varied much in gross appearance; many of the specimens were free from blood and others were quite clear. No periodicity was observable in either of these characteristics. During his stay in the hospital his weight varied between 51 and 47.8 kg. and his hemoglobin fell 22 per cent. Three weeks after admission he had an attack of acute retention, the only one while in the hospital. He was not catheterized and about fifteen minutes later he voided a small amount of very bloody urine containing several fibrinous clots. He was given 0.3 gm. of salvarsan intravenously on October 31. The specimen voided after this contained much less blood and fat than usual. From this time until the date of discharge, November 13, the urine remained free of fat and blood and entirely normal in appearance.

THE FAT CONTENT OF THE URINE

The quantity of fat in chylous urines has been reported several times; the determinations have, however, usually been made on single specimens and without regard to the amount of fat ingested. Dunn[1] reports a case of filarial chyluria in which the determination was made

1. Dunn: Tr. Col. Phys. and Surg., Phila., 1898, xx, 80.

on a single voiding. This contained 2.2 per cent. fat. Young[2] reports two cases in which the fat content of the urine was 1.8 per cent. and 2.6 per cent. Salkowski[3] studied a case and found the urine to contain 0.4 per cent. of fat. Henson[4] has made observations in a case of lymphscrotum on the relation of the fat content of the diet to the amount in the effusion.

The question naturally arises, is the chylous fluid mixed with the urine true chyle or lymph? The composition of lymph is extremely variable, as shown by analyses cited by Hammarsten[5] made on lymph from a fistula in the thigh. This lymph contained 47 parts of fat per 1,000 after the subject had partaken of fat; while on a starvation diet the content varied between 0.6 and 2.6 parts per 1,000. Low[6] reports a case with "chylous" turbid urine in which the urine contained no fat. He therefore introduced the term "lymphuria" to distinguish cases with turbid fat-free urine containing lymph from true cases of chyluria, and believes the pathology of the two conditions to have certain differences. These will be discussed later.

The method in the present case was to collect a sample of each voiding. Of this, 10 c.c. were mixed with about an equal amount of sand and evaporated to dryness on the water bath. The sand containing the urinary residue was then collected in filter papers and extracted with ether for twelve hours in a Soxhlet fat extractor. The ether extract was then transferred to a weighed dish and the ether evaporated by a current of air until the dish had assumed a constant weight. From the amount found in this way the amount of ether-soluble fat in the total specimen of urine was calculated. Table 1 shows the percentage content of fat in the specimens of urine.

On admission the patient was put on the regular house diet, the fat content of which is variable and not accurately known. During this period, which extended from September 14 to September 29, the fat content of single specimens was between 0.2 and 1.2 per cent.

On September 29 he was placed on a fat-poor diet, fat content averaging 13.7 gm. per day. The single voidings during this period, which extended to October 6, averaged 0.35 per cent. fat, and the total average daily output of fat was 3.4457 gm. The daily total fat in the urine in relation to fat intake is shown in Figure 1.

When the house diet was resumed the amount of fat in the single specimens showed marked increase, rising to between 1 and 1.4 per cent. The effect of the transition from house diet to fat-poor diet

2. Young: Jour. Trop. Med. and Hyg., 1914, xvii, 2.
3. Salkowski: Berl. klin. Wchnschr., 1907, xliv, 51.
4. Hensen: Arch. f. d. ges. Physiol., 1875, x, 94.
5. Hammarsten: Text-Book Physiol. Chem., New York, Ed. 5, 1910, p. 252.
6. Low: Jour. Lond. Sch. Trop. Med., 1912, 1, 243.

Fat Content of Urine in Case of Hematochyluria

Date	Time of Voiding	Fat, %
Sept. 14	4:00 p. m.	0.962
	6:40 p. m.	0.404
	9:20 p. m.	1.124
Sept. 15	1:00 a. m.	0.124
	5:15 a. m.	0.402
	9:15 a. m.	0.562
	12:45 p. m.	0.540
	4:50 p. m.	0.192
	10:30 p. m.	0.401
Sept. 16	6:00 a. m.	0.340
	12:00 noon	0.452
	8:00 p. m.	0.213
	10:15 p. m.	0.382
Sept. 18	4:00 a. m.	0.223
	6:30 a. m.	0.852
	12:50 p. m.	0.103
	2:20 p. m.	0.482
	3:00 p. m.	0.529
	6:00 p. m.	0.925
Sept. 19	1:00 a. m.	0.437
	8:30 a. m.	1.110
	1:00 p. m.	1.282
	7:30 p. m.	0.550
Sept. 20	3:00 a. m.	0.649
	7:30 a. m.	0.710
	12:00 noon	1.217
	2:00 p. m.	1.167
	4:20 p. m.	0.747
Sept. 21	8:00 a. m.	0.472
	12:00 noon	0.547
Sept. 22	3:00 a. m.	0.500
	8:30 p. m.	0.610
Sept. 23	1:00 a. m.	0.617
	6:00 a. m.	0.582
	11:25 a. m.	0.462
	2:30 p. m.	0.550
	8:00 p. m.	0.623
Sept. 24	5:00 a. m.	0.410
	11:30 a. m.	0.392
	4:00 p. m.	0.426
	8:00 p. m.	0.561
	11:00 p. m.	0.378
Sept. 25	2:00 a. m.	0.403
	10:00 a. m.	0.801
	12:00 a. m.	0.675
	3:15 p. m.	0.706
	6:10 p. m.	0.458
Sept. 26	1:00 a. m.	0.456
	2:00 a. m.	0.495
	3:30 a. m.	0.561
	8:30 a. m.	0.840
	9:20 a. m.	0.661
	12:30 p. m.	0.919
	3:30 p. m.	1.122
	9:50 p. m.	0.389
Sept. 27	4:00 a. m.	0.218
	9:45 a. m.	0.967
	1:00 p. m.	1.673
	8:00 p. m.	0.989
Sept. 28	2:00 a. m.	0.810
	4:50 a. m.	0.590
	9:30 a. m.	0.786
	12:40 p. m.	0.869
	3:00 p. m.	1.013
	6:00 p. m.	1.611
Sept. 29	2:45 a. m.	0.704
	4:40 a. m.	0.898
	10:40 a. m.	1.342
	3:00 p. m.	0.365
	3:40 p. m.	0.306
	8:40 p. m.	0.422
Sept. 30	1:45 a. m.	0.256
	4:25 a. m.	0.144
	8:45 a. m.	0.234
	11:30 a. m.	0.212
	12:00 noon	0.254
	3:30 p. m.	0.352
	5:30 p. m.	0.295
	7:40 p. m.	0.462
	10:40 p. m.	0.412
	4:00 a. m.	0.482
	9:00 a. m.	1.251
	11:00 a. m.	0.506
	2:30 p. m.	1.022
	4:00 p. m.	0.426
	5:45 p. m.	0.320
	10:00 p. m.	0.436
Oct. 9	2:00 a. m.	0.366
	4:00 a. m.	0.340
	7:40 a. m.	0.686
	2:45 p. m.	0.864
	4:00 p. m.	0.380
Oct. 10	10:20 a. m.	0.754
	11:00 a. m.	0.730
	12:30 p. m.	0.624
	5:00 p. m.	0.782
	9:45 p. m.	0.648
Oct. 11	1:45 a. m.	0.386
	9:30 a. m.	0.863
	12:00 p. m.	0.704
	1:35 p. m.	0.986
	3:00 p. m.	0.742
	4:45 p. m.	0.622
	6:00 p. m.	1.060
Oct. 12	2:15 a. m.	0.384
	4:00 a. m.	0.320
	10:00 a. m.	0.976
Oct. 13	10:00 a. m.	0.850
	11:30 a. m.	0.682
	12:45 p. m.	0.950
Oct. 14	4:00 a. m.	0.446
	6:30 a. m.	0.376
	11:20 a. m.	1.381
	11:40 a. m.	0.741
	3:10 p. m.	0.864
	9:20 p. m.	0.446
Oct. 15	4:00 a. m.	0.360
	9:15 a. m.	1.061
	12:50 p. m.	1.204
	3:45 p. m.	0.861
	6:20 p. m.	0.799
	9:00 p. m.	1.627
Oct. 17	3:30 a. m.	0.750
	4:45 p. m.	0.940
	6:30 p. m.	0.898
	11:30 p. m.	1.151
Oct. 18	4:00 a. m.	0.838
	10:45 a. m.	1.202
	2:40 p. m.	1.550
Oct. 19	4:00 a. m.	0.759
	7:15 a. m.	1.181
	12:00 noon	1.291
	1:00 p. m.	1.442
	8:30 p. m.	0.788
Oct. 20	4:00 a. m.	1.061
	10:00 a. m.	0.986
	12:35 p. m.	0.686
	9:50 p. m.	1.101
Oct. 21	2:00 a. m.	0.506
	8:00 a. m.	1.202
	3:20 p. m.	1.102
Oct. 22	2:00 a. m.	1.204
	6:00 p. m.	1.308
Oct. 23	9:10 a. m.	1.011
	10:30 p. m.	0.986
	3:00 p. m.	0.704
Oct. 24	8:00 a. m.	0.986
	11:00 a. m.	0.630
	11:40 a. m.	0.580
	2:15 p. m.	0.986
	4:00 p. m.	0.543
	6:00 p. m.	1.104
Oct. 25	4:00 a. m.	0.462
	2:15 p. m.	1.702
	5:00 p. m.	0.408
	11:00 p. m.	0.804
Oct. 26	6:45 a. m.	0.386
	9:40 a. m.	0.860
	11:30 a. m.	0.582
	1:20 p. m.	0.940

FAT CONTENT OF URINE IN CASE OF HEMATOCHYLURIA—(*Continued*)

Date	Time of Voiding	Fat, %
Oct. 1	1:00 a. m.	0.356
	4:00 a. m.	0.234
	8:05 a. m.	0.538
	10:30 a. m.	0.426
	2:15 p. m.	0.469
	3:15 p. m.	0.340
	4:33 p. m.	0.286
	9:30 p. m.	0.450
Oct. 2	5:30 a. m.	0.380
	12:00 noon	0.320
	2:30 p. m.	0.452
	7:00 p. m.	0.522
Oct. 5	4:00 a. m.	0.282
	11:00 a. m.	0.342
	1:00 p. m.	0.424
	8:20 p. m.	0.402
Oct. 6	2:00 a. m.	0.262
	2:00 p. m.	1.207
	6:00 p. m.	1.402
	10:45 p. m.	0.346
Oct. 7	4:00 a. m.	0.572
	9:30 a. m.	0.856
	1:00 p. m.	1.401
	7:10 p. m.	0.600
Oct. 8	2:30 a. m.	0.421
	2:35 p. m.	0.406
	6:00 p. m.	1.023
	8:20 p. m.	0.742
Oct. 27	5:00 a. m.	0.420
	7:00 p. m.	0.923
Oct. 28	4:45 a. m.	0.436
	1:55 p. m.	0.906
	2:25 p. m.	0.604
	11:30 p. m.	0.486
Oct. 29	2:00 a. m.	0.789
	11:00 a. m.	0.920
	4:30 p. m.	0.969
	8:25 p. m.	1.128
Oct. 30	8:30 a. m.	0.729
	3:30 p. m.	0.001
Oct. 31	1:45 a. m.	0.719
	2:00 p. m.	0.121
Nov. 1	5:00 a. m.	0.098
	8:00 a. m.	0.006
	9:00 p. m.	0.003
Nov. 2	4:00 a. m.	None
	9:00 a. m.	None
	7:00 p. m.	None
	10:00 p. m.	None

and vice versa on the percentage of fat in single voidings of urine is shown in Figure 2.

The diet next used was one calculated to contain protein 150 gm., carbohydrate 300 gm., and fat 80 gm. Actual estimation of the fat showed this to be 78.5 gm. The amount of fat in the food refused each day was calculated and this subtracted from 78.5 gave the amount actually ingested, as shown in Figure 3. It will be seen from this illustration that the amount of fat in the urine is not strictly proportional to the amount in the food, that is, variations in the intake do not cause proportional variations in the output. During this period, October 15 to 31, the average daily intake was 66.39 gm. and the average daily output was 6.451 gm. The average content of single voidings was 0.9 per cent.

A consideration of these figures would seem to indicate that there are other determining factors besides the amount of fat in the food; for when the amount of fat ingested was increased almost five times, that is, from 13.7 gm. to 66.39 gm., the amount in the urine was not quite doubled, 3.445 gm. to 6.451 gm. per day. Part of this discrepancy might result from faulty absorption when taking a fat-rich food.

In this particular case there was no observable difference between the night and day specimens as regards fat content, that is, the position of the body did not seem to be a factor. Charteris,[7] however, records a case in which chyluria could be induced at will by giving the patient milk to drink, and causing him to remain supine for a short while. The condition was of at least ten years' duration.

7. Charteris: Lancet, London, 1911, ii, 1011.

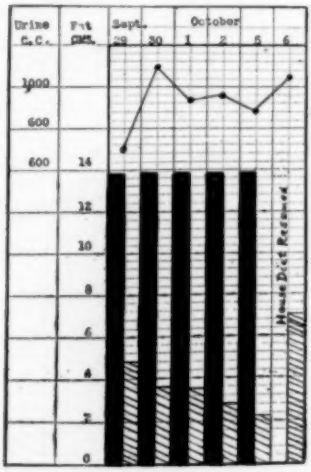

Fig. 1.—The total daily amount of fat in the urine while on a diet low in fat. Solid black columns indicate approximate daily fat intake; cross-hatched columns indicate daily fat output in the urine. Dots joined by line indicate daily amount of urine.

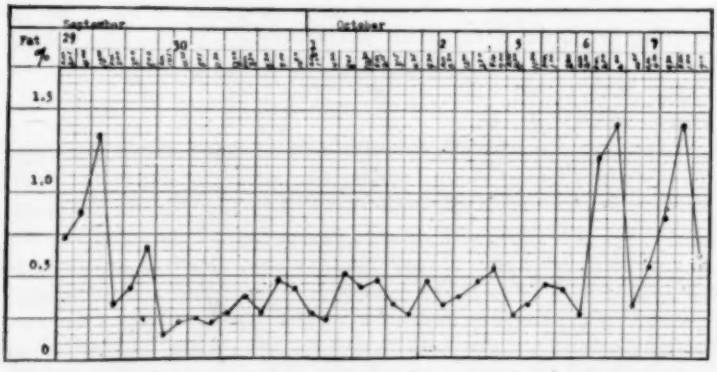

Fig. 2.—The changes in percentage of urinary fat in single voidings on transition to and from a fat-poor diet. The dots represent the percentage of fat in single voidings. On September 29 a diet low in fat was begun and on October 6 it was discontinued for a diet fairly rich in fat.

The daily fluctuations of chyluria and clear urine observed in some cases are explained by Magnus-Levy[7] on a mechanical basis. In about a third of the cases cited by him the urine was turbid for the greater part of the day, and in the majority of the remainder only at night, or when the patient was in a reclining posture. A few patients presented chyluria only when in the upright position. These variations have been explained by assuming that the valves in the lymphatics are

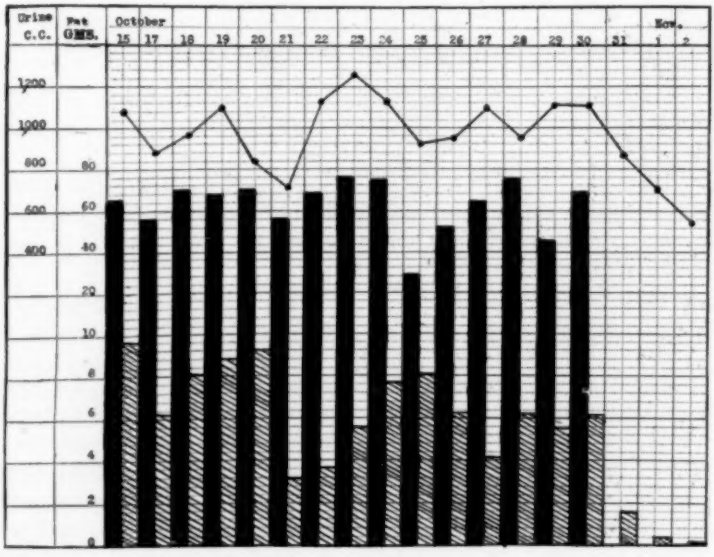

Fig. 3.—The relation of the total daily urinary fat to the amount ingested while on a diet relatively high in fat. Solid black columns indicate approximate daily fat intake; cross-hatched columns indicate daily fat output in the urine. Dots joined by line indicate daily amount of urine.

insufficient, when the body is in a certain position, so that the communication between the lymphatic varix and the urinary system is patent. Other factors which suggest themselves, but have not, I think, been demonstrated in their exact relation to the degree of chyluria, are the size of the fistula into the urinary tract, the hydrostatic pressure causing the emptying of the lymph into the urinary system, and the damming back of the chyle from the thoracic duct.

8. Magnus-Levy: Ztschr. f. klin. Med., 1908, lxvi, 482.

PATHOLOGY

Opportunities to study the pathology of chyluria have not been numerous. As long ago as 1862 Carter[9] suggested that the condition was due to the rupture of a varicose lymphatic vessel into the bladder. That this is a cause is shown by the case reported by Havelburg.[10] The patient had had chyluria for six months, and filaria had been found in the blood and urine. Necropsy revealed a large tumor, extending from the left kidney to the bladder. It consisted of many loculi and its contents were chylous. The upper and left walls of the bladder were in intimate contact with the tumor. On opening the bladder there was found a fistulous opening from which chylous fluid could be squeezed.

Confirmatory of this view is the case of a Japanese who consulted Pope[11] because of turbid urine. On cystoscopic examination a fistula large enough to admit a ureteral catheter was seen near the base of the trigonum, discharging chylous urine. Further examination showed that the urine did contain fat. No filaria were found and there was a history of probable psoas abscess in infancy, which, it was thought, might have been the causative factor.

That the above condition is not essential to the production of chyluria and is, indeed, absent in the majority of instances is shown by several cases which may be cited in some detail.

In the well-known case of MacKenzie[12] the whole of the space between the kidneys was occupied by a mass of varicose lymphatics, which extended down to the iliac vessels. The receptaculum chyli began in two large lymph sinuses about the size of a pencil, one from each side of the aorta. Near the aortic opening in the diaphragm it was joined by a third large lymphatic sinus. The thoracic duct was sinuous and much pouched for three or four inches, varying in diameter from ⅜ to ½ inch, pervious part of the way, filled above with a loose clot. At its termination it passed through a mass of lymphatic tissue, was pervious and about the size of a crow's quill. Although the varix closely embraced the urinary tract, no fistulous communication was observed. Finding the kidney greatly dilated, MacKenzie thought it probable that within the kidney between the lymphatic and urinary apparatus there was a communication by which chylous lymph entered the urine. The bladder appeared to be normal. Chyle and lymph had, however, entirely disappeared from the urine three months before death.

9. Carter: Med.-Chir. Tr., London, 1862, xlv, 189.
10. Havelburg: Arch. f. path. Anat., 1882, lxxxix, 365.
11. Pope: California State Jour. Med., 1909, vii, 285.
12. MacKenzie: Tr. Path. Soc., London, 1882, xxxiii, 394.

Manson[13] records the postmortem findings in a case of filarial chyluria. The thoracic duct commenced below in an ill-defined tumor, situated in front of the vertebrae and below the diaphragm; the thoracic duct was enlarged throughout, though variously, its diameter being equal to the little finger below and to a goose quill above. The upper two or three inches of the duct, with its venous outlet, were quite occluded, below this the duct was patent and its lumen was occupied throughout its length by a firm thrombus, which in its upper extent was closely and almost structurally connected with the duct, but below, where the latter was wider, it was loose and easily detachable. The mass in which the thoracic duct commenced extended downward as far as the right iliac fossa and closely embraced one of the ureters. The kidneys, ureters and bladder were found to be quite healthy and no communication whatever could be found between them and the tumor. The latter on section was tough and fibrous and showed here and there gaping vessels with the walls cut across, which were probably dilated lymphatics.

Low[6] reports a case in which the pathologic findings were quite different from those described above. In his case the thoracic duct was entirely normal, as was also the receptaculum chyli. The kidneys were somewhat enlarged and on section exuded hemolymph. The lymphatics of the kidneys and pelves were in a varicose condition. In a lymphatic of the pelvis of the kidney a calcified filaria was found surrounded by dense fibrous tissue. The left ureter with its lymphatics was uniformly dilated and thickened. No cause of obstruction was found. The bladder was distended, hypertrophic, and contained hemolymph and clots. From its wall there were villus-like projections of varicose lymphatics. Low is of the opinion that in his case there was a leakage of lymph from the left kidney, left ureter, the bladder and probably from the right kidney, in which the condition was less marked. This wide distribution indicated an extensive disturbance or blockage of the lymphatic stream going to the thoracic duct. No obstruction was found, but the conclusion was arrived at that filariae act as an intense irritant producing fibrosis and consequent obstruction. The urine in this case though turbid was free from fat and led to the introduction of the term "lymphuria." The pathologic explanation of this, Low thinks, lies in the fact that in this case obstruction was below the thoracic duct, that is, in the efferent renal lymphatics. For chyle to reach the lymphatics around the bladder there must be regurgitation in the thoracic duct and such regurgitation could not take place if the duct and its venous outlet were patent.

That extensive fibrosis in the region of the hilus of each kidney might produce obstruction is evident when it is recalled that there are

13. Manson: Davidson's Hygiene and Diseases of Warm Climates, p. 814.

normally only four to seven efferent lymphatic trunks which leave the hilus and run along with the renal artery and vein, according to Poirier and Cuneo,[14] to end principally in the juxta-aortic glands of the corresponding side and accessorily in the preaortic glands. After passing through these series of glands the efferents of the right side pass through the right pillar of the diaphragm and terminate in the thoracic duct. On the left side the efferents pass through the left pillar of the diaphragm and also join the thoracic duct. Obstruction of the terminal trunks would lead to gradual dilatation and eventual rupture. That chyluria is not more frequent in cases of lymphatic varix with partial obstruction seems to indicate that the anastomoses are ordinarily sufficient to drain the chyle away from the urinary tract.

SUMMARY

In a patient with chyluria the fat content of the urine varied markedly with the fat content of ingested food. On a fat-poor diet it averaged 0.35 per cent. and on the house diet it rose to from 1 to 1.4 per cent. With diet containing a daily average of 66.39 gm. of fat the average daily output of fat in the urine was 6.45 gm. The amount of fat in the urine, however, did not increase in direct proportion to the amount of ingested fat. In some cases chyle escapes directly into the bladder or ureter through a fistulous opening; in other cases the chyle finds its way into the urine within the kidney. In some cases the fluid entering the bladder is true chyle, in others it is lymph.

14. Poirier and Cuneo: Treat. Human Anat., The Lymphatics, Leaf's Translation.

METABOLISM STUDIES OF ANGIONEUROTIC EDEMA *

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INTRODUCTION

The subject of this investigation is a woman suffering at irregular intervals from attacks of transient edema, sometimes with convulsions, and with a persistent but variable eosinophilia. She has been under observation, more or less constantly, in the wards of the university hospital for a period of eight years and has been during that time the subject of many detailed studies, planned with the object of throwing some light on the etiology or exact nature of her condition. These, however, have yielded no specific information and the condition remains diagnosed only as an obscure type of angioneurotic edema. The present studies are an attempt to demonstrate any changes which may have occurred concomitantly with the attacks of edema, and include a determination of nitrogen utilization; an estimation of the acid-base relations of the body fluids; a study of the chlorid excretion; and uncompleted desultory studies of calcium and fat metabolism and of respiratory exchange.

CLINICAL NOTES

The patient, a woman, now 54 years of age, married and without children, was admitted to a medical ward of the hospital on the service of Dr. Alfred Stengel on June 27, 1908, in a state of unconsciousness following her first convulsion. She had, however, been having monthly attacks of swelling about the eyes from February of the preceding year. The menses had stopped in September of that year.

Since June, 1908, she has had convulsions and edematous attacks at irregular intervals and has spent the greater part of each year in the hospital. She is not confined to bed except during and for a few days following her more severe attacks. Sometimes an attack consists of a single brief convulsion and again of a number coming on without warning and in rapid succession, the type of the convulsion being clonic and involving first the face and then the body generally. Edema of the circumocular areas, the arms, the chin, and the anterior neck frequently precedes, accompanies, or immediately follows these seizures, but either the edema or the convulsions may occur independently. A typical attack of edema appears at first as a slight puffiness about the eyes or a little firmness of the inner aspects of the upper forearms. These parts are often quite itchy and there are sometimes associated urticarial manifestations elsewhere. In the course of a few days the swellings reach their height and then gradually subside. At their height an increase in the circumference of the arm above and below the elbow of from 2 to 4 cm. has often been demonstrated. At

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such times also there is an increase in the patient's weight of from 4 to 8 pounds. One attack may immediately follow another, but as a rule there is an interval of several weeks.

The urine constantly shows a trace of albumin and a few hyaline and granular casts. Its amount is decreased before an attack of marked swelling, and subsequently rises after the cessation of the attack. In an interval between attacks the phenolsulphonephthalein elimination in two hours after intravenous injection was 50 per cent.; during an attack it was 35 per cent. The urea of the blood equals 28 mg. in 100 c.c. of blood. The leukocyte count of the blood varies between normal and 44,000. An eosinophilia is always present, the percentage varying from 27 to 85. Numerous blood pressure studies show that there is a lowering of the readings with the onset of the swellings and that convulsions are most likely to occur during this depression. The systolic pressure varies from 115 to 187 mm. of mercury and the diastolic from 65 to 85. Wassermann tests were twice negative. Repeated examinations of the feces for intestinal parasites have yielded no information.

METHODS

The patient was under observation in a metabolic ward of the hospital during these studies, the details of her diet and the collection of her excreta being supervised by a nurse whose duties were confined to metabolism cases. The diet varied in the several periods of our study, and in the intervals she was permitted the full ward diet. In Periods 1, 3, and 4 the Folin diet[1] was used; in Period 2 a diet of carbohydrates and fats with a minimal amount of protein was employed. In Periods 5 and 6, respectively, a salt free and a high salt diet were given. The urine was collected under toluol and at the end of each 24 hours transferred from the ice box of the ward to that of the laboratory. The feces were marked off into periods by means of carmine.

The total nitrogen of the urine and of the food and feces was estimated by the Kjeldahl-Gunning method. Ammonia was determined by the Folin method and sodium chlorid by the technic of Volhard-Arnold. The determinations of the total acidity of the urine were made by the method of Palmer and Henderson.[2]

RESULTS AND COMMENT

The Nitrogen Metabolism.—In the periods in which the total nitrogen metabolism was studied (Table 1) the Folin diet was employed because it has been standardized and comparisons of results can therefore be made with known figures. It will be observed that in each period there is a positive nitrogen balance. This nitrogen retention, while never very marked, is greater in the two periods which include attacks of edema (Periods 1 and 4a) than in the two normal

1. Folin, O.: Approximately Complete Analyses of Thirty "Normal" Urines, Am. Jour. Physiol., 1905, xiii, 45.

2. Henderson, L. J., and Palmer, W. W.: On the Several Factors of Acid Excretion, Jour. Biol Chem., 1914, vii, 305.

TABLE 1.—METABOLISM IN ANGIONEUROTIC EDEMA

Period	Date	Nitrogen Intake, Gm.	Urine						Feces, Nitrogen, Gm.	Nitrogen Balance	Clinical Notes
			Volume, C.c.	Total Nitrogen, Gm.	Ammonia, Gm.	NH_3 in N/10 Acid	Acidity in N/10 Alk.	NH_3 plus Acidity			
1	1915 10/ 1	17.30	1,950	14.96	0.39	...	...	...	1.76	+0.67	Marked edema
	10/ 2	17.42	1,460	14.14	0.32	...	...	...	1.76	+1.52	
	10/ 3	17.39	1,240	14.63	0.49	...	...	...	1.76	+1.00	
	10/ 4	17.31	1,560	14.90	0.62	...	...	...	1.76	+0.65	
	10/ 5	17.04	1,375	14.19	0.62	...	...	...	1.76	+1.09	
	10/ 6	18.02	1,760	14.31	0.66	...	...	...	1.76	+1.95	
	10/ 7	18.39	1,725	13.49	0.66	...	...	...	1.76	+3.14	
	10/ 8	17.07	1,640	13.49	0.54	...	...	...	1.76	+1.82	
	Average	17.50	1,588	14.26	0.54	...	...	...	1.76	+1.48	
2a	12/ 8	15.75	2,600	10.54	0.41	249	364	613	0.93	+4.28	Convulsion, no edema
	12/ 9	0	800	8.40	0.33	200	260	460	0.93	−9.43	
	12/10	16.67	710	10.39	0.41	252	330	582	0.94	+5.35	
	12/11	17.12	1,240	15.78	0.62	376	480	856	0.94	+0.40	
	Average	12.38	1,352	11.30	0.44	269	358	628	0.93	+0.15	
2b	12/12	17.30	1,630	14.44	0.53	320	510	830	1.32	+1.44	Normal period
	12/13	18.60	1,700	16.05	0.53	330	510	830	1.32	+1.23	
	12/14	17.70	1,800	16.65	0.62	376	560	936	1.33	−0.28	
	12/15	16.90	1,730	16.43	0.53	332	500	832	1.32	−0.85	
	Average	17.60	1,737	15.89	0.55	337	520	857	1.32	+0.38	
4a	1916 1/27	17.2	1,040	13.06	0.59	360	460	820	1.59	+2.55	Marked edema
	1/28	17.8	1,330	13.60	0.63	384	460	844	1.60	+2.60	
	1/29	17.3	1,480	14.23	0.68	412	500	912	1.59	+1.48	
	1/30	17.7	2,335	14.28	0.63	385	487	872	1.60	+1.82	
	1/31	14.5	1,500	14.20	0.60	364	500	864	1.60	−1.30	
	2/ 1	17.4	1,430	13.49	0.54	328	490	818	1.60	+2.31	
	Average	17	1,519	13.81	0.61	372	483	854	1.60	+1.58	
4b	2/ 4	17.3	2,175	16.32	0.61	370	575	945	1.26	−0.28	Normal period
	2/ 5	18.5	1,625	16.24	0.56	340	550	890	1.26	+1.00	
	2/ 6	18.2	1,450	15.12	0.56	340	500	840	1.27	+1.81	
	2/ 7	18.3	1,770	16.32	0.67	412	530	942	1.27	+0.71	
	Average	18.1	1,775	16	0.60	365	539	904	1.26	+0.81	

periods (3b and 4b). In the period which includes a convulsion (3a) the nitrogen retention is a little less even than for the two normal periods. It would appear, therefore, that a convulsive seizure does not interfere with the utilization of nitrogen, whereas an edematous attack does to a limited extent.

The Acid and Ammonia Excretion.—The daily estimations of the total acidity of the urine in the normal periods (3b and 4b) are quite similar. For the intermediate period, which included a marked attack of edema, the figures are only slightly lower. It would seem that this decrease is too slight to be considered of importance in relation to the reaction of the body fluids. Furthermore, this decrease in the acid output of Period 4a is associated with a slightly greater ammonia excretion than occurs in the normal periods. It is the sum of these two factors that represents the efficiency of the kidney in retaining alkali and we must look, therefore, to this figure for any influence on the reaction of the body fluids. In order to express these two factors in the same units the amount of the ammonia excretion is expressed in the number of cubic centimeters of tenth-normal acid necessary to neutralize it, as well as in the usual way, just as the total acidity is expressed in the number of cubic centimeters of tenth-normal alkali necessary to neutralize it. The sum of the two is presented in a separate column, and it will be noted that the variation between the figures for the normal periods and for the period of edema are negligible. In Period 3a, during which there was no edema, but in which a convulsive seizure occurred, there is some reduction in the total acidity, but on the day of the convulsion no food was taken, and consequently the figures are misleading.

The Chlorid Excretion.—Table 2 shows in a number of attacks a clear-cut fall in the sodium chlorid excretion during the several days preceding and including the development of edema. In each instance on the day of the most marked edema the chlorid figures were rising and subsequently they were above normal. In Periods 1, 3, and 4 the salt intake was constant and the relatively low excretion figures for the several days preceding the height of the edema therefore indicate clearly a retention of chlorids. This is further emphasized by the large amounts of chlorid excreted during the periods of subsidence of the edema.

In Period 2, although the amount of intake was not definitely controlled, the diet was sufficiently constant, as indicated by daily determinations of the urinary nitrogen, to exclude the possibility that irregularity in diet could account for the marked diminution in the sodium chlorid excreted on the four days previous to the height of the edema. In Period 5 the patient was on a salt-free diet, and although a convulsion with a little edema did occur, the latter was practically

TABLE 2.—Chlorid Excretion During Attacks of Angioneurotic Edema

	Period 1, Folin Diet			Period 2, Low Protein Diet			Period 3, Folin Diet			Period 4, Folin Diet			Period 5, Salt-Free Diet			Period 6, High-Salt Diet		
	Date	Urine, Vol., C.c.	Urine NaCl, Gm.	Date	Urine, Vol., C.c.	Urine NaCl, Gm.	Date	Urine, Vol., C.c.	Urine NaCl, Gm.	Date	Urine, Vol., C.c.	Urine NaCl, Gm.	Date	Urine, Vol., C.c.	Urine NaCl, Gm.	Date	Urine, Vol., C.c.	Urine NaCl, Gm.
	1915			1915			1915			1916			1916			1916		
	10/ 1	1,950	9.2	11/ 3	1,400	5.2	.. ..	.....	....	1/24	1,740	9.6	....	.....	....	....	.....	....
No edema...	10/ 2	1,460	7.2	11/ 4	1,080	3.6	.. ..	.....	....	1/25	1,320	6.6	....	.....	....	....	.....	....
	10/ 3	1,240	6.8	11/ 5	1,020	2.0	.. ..	.....	....	1/26	1,025	6.6	....	.....	....	....	.....	....
Edema developing..	10/ 4	1,560	8.4	11/ 6	1,200	2	12/ 4	760	3.6	1/27	1,040	7.2	3/21	850	2.3	....	.....	....
	10/ 5	1,375	8	11/ 7	1,300	3	12/ 5	1,100	7.6	1/28	1,330	9.6	3/22	1,225	1.4	4/26	540	9
Edema at height.....	10/ 6	1,700	10	11/ 8	1,080	5.6	12/ 6	1,500	10.6	1/29	1,480	9.8	3/23*	1,280	1.6	4/27†	870	14.5
Edema subsiding.....	10/ 7	1,725	10.4	11/ 9	1,375	8.2	12/ 7	2,700	19.4	1/30	2,335	14	3/24	390	1	4/28	960	14.4
	10/ 8	1,640	10.	11/10	1,520	9.4	12/ 8	2,600	14.3	1/31	1,500	11.4	3/25	770	0.7	4/29	1,080	18

* Convulsion; edema extremely slight.
† Convulsion; edema very marked.

negligible. The patient was on this diet for a number of weeks and at no time did any appreciable swelling develop, although convulsions did occur. In Period 6 she was put on a high salt diet (the usual hospital diet with 10 gm. of salt added) and a convulsion occurred and associated with it was a marked edema. Unfortunately the figures which we present include but one day before this period of pronounced swelling; the excretion for that day, however, shows a preliminary retention.

Other Data.—In addition to the results presented in our tables we have made some investigations of the calcium and fat metabolism, but these studies have not been sufficiently completed to justify their publication. We may state, however, that they point toward normal fat and calcium utilization. Also some investigations of the carbon dioxid content of the blood and the alveolar air and of the chlorid content of the blood plasma have been made for us in this patient and found normal.

CONCLUSIONS

There is some evidence, as is indicated in our clinical notes, of an impaired renal function in this patient, and it is impossible to say to what extent this disturbance may have influenced our results. We feel justified, nevertheless, in presenting the following conclusions:

1. The attacks of swelling in an individual with an obscure type of angioneurotic edema are associated with a retention of nitrogen, which is greater than appears in intervening periods.

2. The excretion of acid bodies, so far as it can be determined by estimations of the total acidity and ammonia excretion in the urine, is not altered during these attacks.

3. A disturbance of the sodium chlorid metabolism is evidenced by a reduced elimination during the three or four days preceding the maximum swelling and by a corresponding excess of excretion afterward.

4. The convulsions from which the patient suffers are not associated with a nitrogen balance which is significantly different from that of normal periods, and there is no coincident change in the acid, ammonia, or sodium chlorid excretion.

5. A low chlorid intake appears to have a beneficial effect on the attacks of edema, but does not influence the convulsions.